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# Leaf stoichiometry of deciduous tree species in different soils exposed to free-air $O_3$ enrichment over two growing seasons

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**Abstract:**

The effects of elevated $O_3$ in different soil conditions on foliar elements stoichiometry were investigated in 3 native tree species with free-air enrichment systems in northern Japan over two growing seasons. Essential elements (Mg, K, Ca, Mn, Fe, Ni, P, N) and two non-essential elements (Cr, Al) were analyzed in leaf samples obtained from 6 different treatments of $O_3$ and soils at 5 collection times from 2014 to 2015. In this study, relationships among the foliar elements within each species were investigated and negative correlations between foliar N and the metal elements were observed in birch (*Betula platyphylla* var. *japonica*). From the differences of foliar contents as well as their re-translocation rate, beech (*Fagus crenata*) with determinate shoot growth pattern was rather more sensitive to $O_3$ stress on foliar contents, meanwhile oak (*Quercus mongolica* var. *crispula*) was possibly susceptible to $O_3$ on dynamics of immobile elements. Soil nutrients have distinct impacts on retranslocation rate of K, Fe, and P. Principal component analysis revealed that Mn and K can become indices in assessing the $O_3$ and soil effects in both short and long term monitoring of the growth of these tree species. Our findings are essential in further comprehension to nutrient conservation mechanism in the nutrient dynamics of cool-temperate forests.

**Key words:** Stoichiometry, Foliar nutrients, Free-air ozone, volcanic ash soil, Serpentine soil

## 1. Introduction

Tropospheric ozone, namely ground-level ozone ($O_3$) as the characteristic of strongly oxidizing, is recognized as one of the important widespread atmospheric pollutants, inducing morphological and chemical changes in leaves that adversely affect the vigor and health of forests (e.g. Bussotti et al., 2005; Yamaguchi et al., 2011; Feng et al., 2015). $O_3$ concentrations have been increased in East Asia and it is expected to be continuously enhanced for decades in foreseeable future because of dramatic increases in emissions of precursor gases (e.g. Akimoto 2003; Ohara et al., 2007; Paoletti, 2007; Watanabe et al., 2010). Recently, relatively high $O_3$ concentration has been detected frequently over wide areas of Japan (Wakamatsu et al., 1998; Takeda and Aihara, 2007; Watanabe et al., 2010; Koike et al., 2013).

As the major deciduous broad-leaved tree species native to northern Japan (Koike 1988), Japanese white birch (hereafter birch: *Betula platyphylla* var. *japonica* Hara) is a typical pioneer tree species with heterophyllous shoot development, continuously unfold leaves one by one (Koike, 1995; Wang et al., 2000; Hoshika et al., 2013); Siebold's beech (beech: *Fagus crenata* Blume) is typical determinant shoot growth and high re-translocation ability of foliar nutrients (Shi et al. 2016a). This species can be of help in conservation of soil and maintain the biodiversity in forests (Watanabe et al., 2010); and both have diffuse porous wood. Mizunara oak (oak: *Quercus mongolica* var. *crispula* Ohashi) has ring porous wood and succeeding type shoot development (Kitao et al.

2015; Koike 1988; Shi et al. 2016a;b).

These 3 species play an essential role in cool temperate forests and provide benefits in social, economic and environment (Koike, 1988; Koike et al., 1998). However, forest decline and dieback of beech as well as oak wilt disease symptoms have been markedly observed in Japan that was believed to be likely due to the high $O_3$ concentrations (Kume et al., 2009; Watanabe et al., 2010). Although there was no reduction effect on short-time $O_3$ exposure in birch, long-time $O_3$ exposure may have a negative impact on the growth (Matsumura et al., 2005; Hoshika et al., 2012a). There are big variations in sensitivity to $O_3$ between various species grown in Japan. Among the 3 species, beech with determinate shoot growth pattern is more sensitive to $O_3$ and its nutrient retranslocation is more efficient (Kohno et al., 2005; Shi et al. 2016a). Oak is reported as a tolerant species with a highly capacity of $O_3$ stress according to screening experiments of Kohno et al. (2005) and Yamaguchi et al. (2011).

The effect of elevated $O_3$ concentration (hereafter, $eO_3$) on tree species is also greatly affected by soil conditions due to plants receive most elements from soils and fertilizers (e.g. Agathokleous et al., 2016). For example, beech may become more sensitive to $O_3$ with increasing of nitrogen (N) load in soil (Yamaguchi et al., 2007); the adverse effects of $O_3$ on beech growth become less under chronic mild water-stressed condition (Watanabe et al., 2005). Therefore, we selected 3 different common soil properties that widely distributed in northern Japan as 3 different soil conditions in this study. Brown forest soil (B) which is mostly Cambisols, is a fertile soil type containing sufficient necessary nutrients for plant growth (Kayama et al., 2011; Kim et al., 2011); volcanic ash soil (V) which is deemed strongly acidic soil, contains high contents in aluminum (Al) and iron (Fe) but low in major and secondary nutrient elements (Kayama et al., 2011; Watanabe et al., 2010); serpentine soil (S) is lacking in several necessary nutrients with high ratio of magnesium (Mg)/calcium (Ca) and excessive heavy metals such as nickel (Ni) and chrome (Cr) that is widely known to be an infertile soil type in Japan (Brady et al., 2005; Kayama and Koike, 2015).

Leaf stoichiometry plays an essential role in the evaluation of plants composition, ecosystem function and nutrient limitation, etc. (Elser et al., 2000; Allen and Gillooly, 2009; Wu et al., 2012). Ecological stoichiometry deals with the balance of multiple elements in ecology interactions (Elser et al., 2000; Rong et al., 2015). Several studies have revealed that $eO_3$ may have great impacts on foliar nutrient contents and can accelerate leaf senescence (Ribas et al., 2005; Hoshika et al., 2012b), resulting in an elemental imbalance in plants (Kam et al., 2015; Shi et al., 2016a). On one hand, a frequently observed effect of $eO_3$ is a decline of N acquisition in plants, causes the nutrient deficiencies in leaves and thereby affects foliar physiological and biochemical functions (Karnosky et al., 2007; Yamaguchi et al., 2010). Moreover, there were observations on the reduction of N, phosphorus (P) contents (Cao et al., 2016) and alternation on their allocations dynamics under $eO_3$ treatment (Shi et al., 2016a). On the other hand, there were considerations suggested that the compounds for the biosynthesis of protein were increased by $eO_3$ and the metabolism of N was enhanced through the increased activities of foliar enzymes (Yamaguchi et al., 2007).

Until recently, although many studies of foliar stoichiometry in response to various environmental conditions have been carried out, most of which focus on the elevated $CO_2$ (e.g. Huang et al., 2012) or soil fertilization (e.g. Dordas and Sioulas, 2009; Ostertag, 2010) as an independent treatment and discuss only primary nutrients such as N, P for one specific tree species (e.g. Elser et al., 2000; Zhang et al., 2010; Huang et al., 2012; Rong et al., 2015). A handful of studies were about the effect of $eO_3$ on foliar stoichiometry with secondary nutrients or trace

elements: fewer studies were conducted with free-air enrichment system for field-grown trees; much-limited studies were concerned with combined effects of $eO_3$ and soil conditions on various foliar elements stoichiometry among different species.

In a previous work, we studied the re-translocation of foliar nutrients compared with only mid-summer and leaf senescence period in deciduous broad-leaved trees with typical shoot development in responses to $eO_3$ and soils (Shi et al. 2016a). From previous results, it is expected that re-translocation of the studied elements under poor nutrition soil without toxic substances are likely more effective in beech species due to its determinate shoot growth pattern and the foliar stoichiometry in beech species is supposed to be more sensitive to $eO_3$. In this study, we further present a biochemical and physiological analysis of the stoichiometry of 10 foliar elements (N, P, Potassium: K, Ca, Mg, Manganese: Mn, Fe, Cr, Al, and Ni) in 3 tree species for longer-time free-air $O_3$ exposure to examine the effects of $eO_3$ alone and together with different soil conditions.

In order to fulfill the achievements, we investigated the stoichiometry issues from following aspects: 1) Effects of $O_3$ or/and soil on foliar element contents in different species, and we expected that elements in oak leaves may have the least effects of $O_3$ since oak is considered to be $O_3$ tolerant in many previous studies (e.g. Kohno et al., 2005; Paoletti et al., 2007; Yamaguchi et al., 2011); 2) Effects of $O_3$ or/and soil on foliar retranslocation rates, and we expected that mineral element retranslocation may be sensitive to the treatment in beech (Shi et al., 2016a); 3) Comparisons among species on elemental retranslocation rate during two growing seasons, and we expected that long-term exposure of $eO_3$ has more adversely influence on foliar elements dynamics, especially for beech species; 4) Foliar elements contents and their relationships, and we expected that various highly correlations among foliar elements and also different contents of elements in leaves for each species may change the elements allocation dynamics and thereby affect the physiological and biochemical function in foliage; 5) Profiling the elements in different species by PCA analysis, and we expected to observe index elements that are able to assess the $O_3$ and soil effects on these species.

Based on the findings, we also discussed on the essential roles of physiological functions for each element in terms of specific traits of each tree species, and establish interactions between the environmental factors and the foliar elements among different species. Our understanding of foliar elements stoichiometry in response of high $O_3$ can be of help to understand elements cycling under elevated $O_3$ world.

## 2. Material and Methods

### *2.1 Plant materials and experimental site*

On mid-July 2014, 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*) which were obtained from Hokkaido Horti-Green Co. Ltd. near Sapporo were planted in field at nursery of Sapporo Experimental Forest, Hokkaido University, in northern Japan (43°04' N, 141°20' E, 15 m a.s.l.), and were exposed to an experimentally enhanced $O_3$ regime with a free-air $O_3$ enrichment system (Shi et al, 2016a) for two growing seasons. All the plants were grown naturally without intentionally irrigation and fertilization. The enrichment system was temporarily closed during the snow period of Sapporo (from mid-December to early-May). Therefore, the $O_3$ exposure system was employed from early-August to mid-November in 2014 and from mid-June to early-December in 2015. The annual mean temperature and total precipitation of Sapporo in 2014 and 2015 were 8.2℃, 1129 mm and 10.3℃, 1331mm respectively.

The experimental sites consist of 6 circular plots, 3 for ambient $O_3$ and another 3 for elevated $O_3$. Each circular plot is 6.5 m in diameter with 5.2 m high dispersal pipes surrounded by (Kim et al., 2011). The distance between the ambient plots and elevated $O_3$ plots was about 20 m. The method of $O_3$ exposure applied in this study is based on the system used at Kranzberg Forest in Germany (Nunn et al, 2002; Werner and Fabian, 2002). Watanabe et al. (2013) described more details about the $O_3$ exposure as well as the plot design in the field station. The target $O_3$ concentration of the elevated $O_3$ plots was 70 ± 7 nmol $mol^{-1}$ during daylight hours (about 7 h $d^{-1}$), while the ambient plots were subjected to daytime $O_3$ concentration of 30 ± 4 nmol $mol^{-1}$.

Each of the experimental system plot was divided into 3 parts and replaced with 3 different typical soils distributed in northern Japan. Brown forest soil (B) which was regarded as control in this study, is native to the nursery of Sapporo Experimental Forest; Volcanic ash soil (V) was taken from the Tomakomai Experimental Forest in southern Hokkaido (Kim et al, 2011); Serpentine soil (S) was taken from the eastern area of Teshio Experimental Forest in northern Hokkaido (Watanabe et al, 2012). The detailed for the design of each free-air enrichment experimental circular plot as well as the distribution of those 3 soils in each plot is shown in Figure 1.

*2.2 Samplings and measurements*

Leaf sampling taken from 3 seedlings per species at each treatment (6 treatments in total) with 3-4 replications was conducted every other month during the snow-free period when the free-air $O_3$ enrichment system was running. Leaf samples were collected totally 5 times within two growing seasons as the different development stages of leaf growth: mid-September and mid-November for first growing season in 2014; mid-July, early-September and mid-November for second growing season in 2015. Samples we collected in September and November are considered respectively as mature leaves with peak and senescing leaves with low nutritional activities in this study. Mature leaves were collected from the first or the second leaf counted from top of a shoot and senescing leaves were collected from the fallen leaves after those shoots gently being shaken. Every time we collected 1-2 sample leaves for the same seedling at each treatment. All the collected leaf samples were dried at 70 ℃ for 5-7 days after rinsing the surface with distilled water and were mill grounded into powder.

Soil cores at the depth of 0-10 cm (100 ml) were collected from each plot (3 cores per the treatment, totally 18 cores) in April 2015 and were air-dried for over one month to evaluate the chemical properties of the soil. After passed through a 2 mm mesh of sieve for a pretreatment, soil samples were prepared for next measurements. In order to determine the soil pH values, 10 g of soil sample was shaken with 25 ml de-ion water for 1 h (Watanabe et al., 2012) and was afterwards measured with a pH meter (HM30V, TOA Electronics Ltd., Tokyo, Japan). After the measurements of pH values, soil samples were dried at 105 ℃ for at least 24 h. In order to extract the exchangeable cations from soil samples for further elemental concentration analysis, 2 g of soil sample was shaken with 40 ml of 1 M ammonium acetate solution for 1 h (Thomas, 1982; Watanabe et al., 2012) and was well filtered to get an extract soil solution.

For the purpose of measurements of the elemental concentrations of Mg, K, Ca, Mn, Fe, Ni, Al, Cr and foliar P, 50 mg leaf samples as well as the extract solutions of soil that obtained in the above step were digested by nitric acid and hydrogen peroxide. Analysis of the elemental concentrations of leaf and soil samples were then conducted with an Inductive Coupled Plasma Mass Spectrometer (ICP-MS, IRIS/IRIS Advantage ICAP, Thermo Fisher Scientific Inc., MA,

USA). For the purpose of measurements of available P in soils, P was extracted with the Bray-2 method as follow: 1g soil sample was shaken with 20 ml extracting solution containing 0.03M ammonium fluoride and 0.1 M hydrochloric acid (Bray and Kurtz, 1945). The extracted solution of P was then determined by molybdenium blue method with ascorbic acid (Murphy and Riley, 1962) using with a spectrophotometer (Gene Spec III, Hitachi, Tokyo, Japan) at the wavelength of 710 nm. The combustion method with a NC analyzer (NC-900, Sumica, Osaka, Japan) was utilized to measure the N concentration in both foliar and soil samples.

Retranslocation rate can be expressed by element resorption efficiency with the formula:

$$\mathrm{ERE} = \frac{\mathrm{Cmature} - \mathrm{Csenescing}}{\mathrm{Cmature}},$$

where ERE is the elements (N, P, K, Ca, Mg, Mn, Fe, Cr, Al or Ni) resorption efficiency (RE). For each specific element X, the element X resorption efficiency is expressed by "XRE" for being better distinguished in this test; $C_{mature}$ is the element concentration of mature leaves which collected in September and $C_{senescing}$ is the element concentration of senescing leaves which collected in November (Huang et al., 2007; Shi et al., 2016a). From this equation, the percentage of elements resorbed prior to leaf abscission as well as the retranslocation rate can be estimated.

*2.3 Soil chemistry*

According to the soil chemical properties shown in Table 1, there was no significant difference in soil pH values as well as the chemical elements contents between the two $O_3$ treatments. As a non-essential element, Cr content in brown forest (B) soil was significantly lower ($p<0.5$) among the 3 soils. As expected, Fe and Al contents in volcanic ash (V) soil were significantly higher ($p<0.5$) than that in brown forest and serpentine soils. Serpentine (S) soil with pH about 6.1, had a significantly higher ($p<0.001$) value than that of B soil and V soil. Besides, Mg, Ni and Cr contents in S soil were about 68.6, 0.33 and 0.11 mg $100g^{-1}$, respectively, significantly higher (Mg, Ni $p<0.001$, Cr $p<0.05$) than that in B soil and V soil. Contrary, S soil contents of K, Ca, Mn, Al and N were significantly lower (N $p<0.01$, others $p<0.05$) compared to the other two soil types. Mg/Ca ratio in S soil was about 0.64 which was the highest among 0.10 in B soil and 0.09 in V soil. Soil content of P did not have significantly differences among the soil types, which was consistent with the results of northern Japan by Kayama and Koike (2015).

*2.4 Statistical analysis*

The distribution of the data was tested for normality with the Kolmogorov-Smirnov test. Two-way ANOVA followed by post hoc Tukey's hsd test was conducted to compare the difference in soil chemical properties among the soil types under different $O_3$ enrichment. Since we collected the same seedlings each time over two growing seasons, a two-way ($O_3$ and Soil) repeated-measures analysis of variance (ANOVA) with collection time or growing seasons as within-subjects variable was utilized to compare the effects of $O_3$ (ambient $O_3$ and elevated $O_3$ concentration) and Soil (brown forest soil, volcanic ash soil and serpentine soil) on foliar contents or retranslocation rate in different time over two growing seasons for each species. Comparison results among the 6 treatments for both foliar contents and retranslocation rates were obtained by general liner model (GLM) analysis or post hoc Tukey's hsd test, after significant result of the GLM based on the

standardized data. In order to assess the retranslocation differences between two growing seasons as well as the species differences within one growing season, one-way repeated-measures ANOVA followed by post hoc Tukey's hsd was applied. For correlation analysis, Pearson correlation coefficients (r) with Student's t test with a conservative p value cut off of 0.001 were applied to identify the significant correlations among foliar elements in three species. Descriptive statistics and the coefficient of variation (CV) were applied to evaluate the elements foliar contents among different species.

The overall foliar contents of 10 elements of 3 species with $O_3$ and soil treatments were profiled by principal component analysis (PCA) in order to reduce the number of variables and screen the data for different treatments by clustering elements with close interactions into novel principal component. The rotation method was the varimax with the Kaiser Normalization based on the Cohen's d. The treatments of $O_3$ and soil were applied as the classification criteria in assessing the relations among them and the elements.

All the statistics analyses were performed with SPSS 21.0 (IBM, NY, USA) and MS EXCEL 2010 to visualize the differences of 10 elements in 3 species under 6 combined factorial treatments of $O_3$ and soils over two growing seasons.

## 3. Results

### *3.1 Variations of leaf-element contents and the element retranslocation with environmental variable*

Table 2 provides statistics summary for the levels of two-way repeated-measures ANOVA on foliar elements contents and their retranslocation rates (ERE) with collection time ("Time") and growing season ("GS") as within-subjects variable, respectively, for the effects of $O_3$ exposure ("$O_3$") and soil types ("Soil") in each species. Foliar contents (Mg, K, Ca, Mn, Al and P) and their corresponding retranslocation rates as well as NiRE and CrRE in these 3 species were detected to have a notable effect in response to either $O_3$ or soil. Leaf Mg, Mn, P contents in birch species (Table 2a) were significantly affected by $O_3$ exposure. The $O_3$ exposure affected also leaf NiRE and PRE in birch though no significant differences were observed among the 6 combined treatments of $O_3$ and soil on NiRE as shown in Table 4a.

In birch species, only leaf K and its KRE were detected to be significantly affected by soil and interaction of $O_3$ x Soil, respectively. In beech species, e$O_3$ effects were detected on foliar contents of K, Al as well as retranslocation rate of Mg, Ca and P (Table 2b). Moreover, leaf Mg, K, Al and KRE, CaRE, PRE were significantly affect by soil types. Foliar Mn and Al, ERE of K and P were detected to be influenced by interactions of $O_3$ x Soil in beech. As for oak species shown in Table 2c, $O_3$ exposure effects were significantly detected on leaf Al, P and their retranslocation rates in addition to MnRE. Although no significant differences were observed between soil and foliar contents, KRE, CaRE, CrRE and PRE were found to be notable affected by soil types. Besides, interaction of $O_3$ and Soil was observed to significantly affect leaf Ca content and KRE, PRE in oak species. In addition, time factors ("Time" or "GS") had markedly affected foliar contents but not retranslocation rates in three species (Table 2). There were no significant effects on interactions of time factors x $O_3$ x Soil for both foliar element contents and retranslocation rates. In this study, we presented the main effects on the parameters and factors that were determined and only the significant results were addressed to be discussed.

### *3.2 Effects of $O_3$ and soil treatments on foliar element content*

Table 3 illustrates the mean foliar element concentrations in birch, beech and oak species and the comparison results among the 6 treatments that combined with $O_3$ (Ambient: amb, Elevated: e) and soil (B, V, S). For birch species, it is apparently from the Table 3a that leaf Mg under serpentine soil as well as Mn under all soil types were dramatically decreased by $eO_3$. Foliar P concentration was dramatically lower at $eO_3$-S than that at amb-V. Table 3b reported significantly more foliar Mg but less K were observed at SE soil condition in beech species. Foliar Al content under S soil was dramatically decreased at ambient but it was significantly enhanced at $eO_3$. Whereas in oak species (Table 3c), leaf Ca content at $eO_3$ was significantly reduced by grown under S soil. We also found that foliar P in oak was significantly affected by $eO_3$ when leaf P was markedly reduced at $eO_3$-V compared with that at amb-S. In addition, foliar element concentration (Table 3) was higher than that in soil (Table 1).

*3.3 Effects of $O_3$ and soil treatments on foliar element resorption efficiency (retranslocation rate)*

Table 4 presents the treatment effects on foliar retranslocation rate of various elements in the 3 tree seedlings. In birch species, KRE and PRE were found to be significantly increased under S soil at $eO_3$ (Table 4a). In beech species, MgRE as well as CaRE was decreased with $eO_3$, especially CaRE was highly significantly decreased at $eO_3$. Moreover, KRE was significantly higher at $eO_3$-B soil than that at amb-V soil in beech species. PRE in beech, similar as it was in birch, was identified to be significantly increased under SE soil at $eO_3$ (Table 4b). In oak species (Table 4c), similar to the results in birch, KRE and PRE were both significantly enhanced at $eO_3$-S soil. Contractively, CaRE was detected to be considerable decreased under S soil at amb. MnRE and PRE were exposed notably higher by $eO_3$ at V soil in our results of oak species. Reduction of AlRE and CrRE were also identified at $eO_3$-S and amb-S, respectively in oak species.

*3.4 Differences of foliar element resorption efficiency (retranslocation rate) during two growing seasons among species*

The results of ERE differences between two growing seasons among the three species are set out in Table 5. Depending on comparison of the effects within one growing season as well as across two growing seasons, the consequences could be divided into 6 groups in terms of the exact same effects on ERE. 1) MgRE and PRE were found to have distinct differences in birch within 2$^{nd}$ growing season, meanwhile had considerable differences in beech across two growing seasons. 2) KRE and MnRE in oak were detected to be significantly different not only within 1$^{st}$ growing season in 2014 but also between the two growing season in 2014 and 2015. 3) CaRE, FeRE and together with AlRE were assessed to have notable differences in birch species within both 1$^{st}$ and 2$^{nd}$ growing seasons, respectively. 4) NiRE in oak was similar to group (2) that had significant differences of across growing seasons but the within one growing season differences were found in 2$^{nd}$ growing season. 5) NRE in birch was comparable to group (3) that was found to be significantly different within both 2014 and 2015, while the major differences across two growing seasons were found in oak species. 6) CrRE in none of the species was observed to have differences either within one growing season or across two growing seasons.

*3.5 Foliar element contents*

Foliar contents of 10 elements in this study varied by 3 orders of magnitude from about 0.02 mg/g for Cr to about 20 mg/g for N, with the general order of Cr < Ni < Al < Fe (Mn ) < P < Mg <

K < Ca < N for these 3 tree species (Figure 2). There were a bit differences on foliar contents of Mn and Fe depending upon species. Descriptive statistics including some main statistical parameters such as mean, standard deviation (SD), maximum and minimum values of foliar elements contents were analyzed, and the results are shown in Table 6. Birch contains more foliar Fe than Mn (Figure 2A) while beech and oak contain more foliar Mn than Fe as shown in the Figure 2B and 2C respectively, which were consistent with the results presented in Table 6. This is probably related to birch species with indeterminate shoot growth pattern.

In order to visualize the species variance influencing each element, coefficient of variation (CV) was presented in Figure 3. The CV of macronutrients in the studied 3 species including N, P, K, Ca and Mg varied <50%, whereas trace elements including Ni and Cr had CVs >100%. The pattern of CV was found to be concordant with the stability of limiting elements: P, K, Ca in birch, N and Ca in beech, as well as K and Ca in oak were < 30%, whereas Al and Fe in birch, Mn and Al in beech and oak were > 50%, and Ni and Cr were approximated to 120% in all species.

*3.6 Relationships among foliar elements*

Leaf elements which are controlled by the homeostatic mechanism are reported to have interaction effects not only within foliar nutrients but also across the species (Baxter et al., 2012; Chu et al., 2015). Therefore, in order to further understand the foliar stoichiometry and better explain the foliar elements contents affected by either $O_3$ or soil type as well as their allocations and dynamics, we investigated the correlations among 10 foliar elements within each species to identify the correlated foliar elements in birch, beech and oak as shown in the whole plots in Figure 4. We also detected a large number of positively correlated foliar elements in all species as shown in Figure 4, however, negatively correlated ones were very limited. In datasets of birch, there were 3 pairs of foliar elements negatively correlated (N-Fe, N-Mn and N-Ca) while in other two species, only N-Al and N-Fe were detected negatively correlated in beech and oak, respectively (Figure 4b and 4c).

*3.7 PCA analysis*

In order to identify the foliar elements associated with the effects of $O_3$ and soil treatments and also screen variables to summarize the data for different treatments across different species, principle component analysis (PCA) was conducted and the results are shown in Figure 5. Ambient $O_3$ with B soil were regarded as control during the analyses. There were 10 foliar elements converted to 2 linearly uncorrelated PCs that in total contributed to 63.2 % of total variance of elements among species. PC1 which can be regarded as the major axis; explained 42.5 % of the total variance and it was dominantly incorporated by beech species and $O_3$ treatment with contribution of 91 % and 25.2 %, respectively. Meanwhile, PC2 explained 20.8 % of total variance and was mainly affected by birch species and soil treatment with contribution of 73.4 % and 14.2 %, respectively.

From PCA analysis shown in Figure 5, PC1 was clearly separated birch species from beech and oak as well as separated combined $O_3$ and soil effects from independent treatment of $O_3$. Therefore, birch and the combined treatments were characterized by their higher foliar N with 75 % contribution loading on PC1, whereas oak and control were characterized by their higher metal leaf contents (Al, Fe, Mn, Ni and Cr with contribution of 78.5 %, 76.3 %, 75.5 %, 72.8 % and 72.2 %, respectively). PC2 was determined by foliar K, P and Mg with the contribution of 78.9 %, 67.4 % and 63.3 %, respectively which were the top 3 highest for soil treatment and birch species.

## 4. Discussion

### *4.1 General analysis of effects of $O_3$ and soil on foliar contents and retranslocation rates*

Foliar mineral content depends on availability and extent of translocation of an element between xylem flow and phloem loading (Gjengedal, 1996); correspondingly, retranslocation is a process of a nutrient element removal from aged plant tissue (e.g. foliage) into other plant organs (e.g. fine roots), providing nutrients for the production prior to senescence (Shi et al., 2016a; Killingbeck, 2004). Foliar contents and their retranslocation rate are closely linked with each other. It is anticipated that those environmental factors or treatments which promote the plant growth also facilitate the retranslocation rate of elements and vice versa (Nambiar and Fife, 1991). As a result, an agreement result was found in our study that the effects of $O_3$ and soil treatments on the retranslocation rate can be detected in those elements basically detected to have significantly treatment effects on foliar contents (Mg, K, Ca Mn, Al and P) among 3 species (Table 3, 4). Therefore, understanding the mechanism of both foliar nutrient contents and their corresponding retranslocation rate are essential to be of help understand the dynamics of nutrient cycling as well as the maintenance of soil fertility in forest ecosystems (Onyekwelu et al., 2006).

Generally speaking, the nutrients re-translocated more on nutrient poor soil sites, manifesting the essential function of retranslocation in the nutrient conservation mechanism (Koike, 2004; Uddling et al., 2006). The amount to retranslocation as well as retranslocation rate can be varied depending on the elements mobility, plant species, and environmental factors such as $O_3$ effect and soil nutrient status (Hagen-Thorn et al., 2004). Among the studied 10 elements, Mg, K, Ni, P and N are known as water soluble elements and are also able to mobile in phloem translocated from old to younger leaves. Contrarily, Ca, Mn, Fe, Al and Cr as the non-mobile elements are move upward via xylem from roots to foliage but poorly mobile in phloem (Zayed and Terry, 2003; Marschner, 2012). Therefore, during leaf senescence, mobile elements are unstably transported out from the senescing leaves and anticipate positive values on retranslocation rates; while non-mobile elements are basically remained in older leaves and result in the deficiencies in younger leaves, expecting negative values on retranslocation rates (Helmisaari, 1992; Marschner, 2012; Shi et al., 2016a).

Within two growing seasons, different treatments of combined $O_3$ and soils had different effects on foliar elements contents in each species. Significant effects were detected in different collection time for all (or almost) foliar elements contents in birch, beech and oak. This is because we collected foliage samples in different development stages of leaf growth and the significant differences detected for foliar nutrients at different leaf stages may due to the different shoot growth pattern of different species (Shi et al., 2016). Soil elements Mg, K, Ca, Mn, Fe, Ni, Cr, Al and N (projected nutrient elements except P) exhibited significant differences among soil types. As the same aged seedlings were planted and developed under the same site conditions, the nutrient differences of surface soil (depth 0–10 cm) are likely due to the elemental function of each species (e.g. Salehi et al., 2013).

### *4.2 Effects of $O_3$ and soil on foliar contents and retranslocation rates in birch species*

As Mg is the central atom of chlorophyll pigment and an essential element for the aggregation of ribosomes (Marschner, 2012) as well as Mn promotes the water oxidation occurrence in photosynthetic oxygen evolution (e.g. Kitao et al., 1998). Both Mg and Mn are the key elements playing important roles in photosynthesis. As leaf Mg and Mn contents were influenced by $eO_3$ exposure in a similar manner (Table 2a) in birch species, lower value of foliar Mg and Mn at $eO_3$

(Table 3a) may associate with the $eO_3$-induced accelerants of foliar senescence and the lower stomata conductance at $eO_3$ (Hoshika et al., 2015). The similar effects of $eO_3$ on Mg and Mn are possibly also attributed to the positive correlations as we identified in birch (Figure 4a). As one of the primary essential elements for plants growth, K must be dissolved into soil water and enters the plant as roots uptake from water (Marschner, 2012). Therefore, foliar K as a highly mobile element in plant was observed significantly affected by soil conditions as shown in Table 2a. Leaf K in birch was decreased with nutrient infertility soil independent of the $O_3$ condition (Table 3b). Contrastively, accumulation of toxic elements (Mg, Ni and Cr) performed higher effects in soils (Table 1). We consider that the major factor for leaf K inhibition of birch species grown in S soil was the toxic elements accumulation in the infertility soil. Previous research reported that a sufficient N habitat (e.g. B soil) induced birch species greater resistance to $O_3$ (Pääkkönen and Holopainen, 1995), which may result to increased nutrient concentration in leaf. Considering the significant effect of $O_3$ was found in foliar P (Table 2a), a possible explanation for the reduced results in foliar P might because of the higher sensitivity effects to $O_3$ for birch species. Moreover, the enhanced KRE and PRE at $eO_3$-S (Table 4a) were in agreement with the study of Koike (2004), reported that the nutrient retranslocation in late successional seedlings can be increased at nutrient deficiency soil. Consider about the effects (or combined effects) of $eO_3$, there is a possibility that both KRE and PRE were increased under nutrient limit soil (Table 4a) to enhance the competitive at plant-level so as to overcome the stressed environment (e.g. $eO_3$) (Aerts and Chapin, 2000). Similar explanation can be also applied on enhanced result of PRE in beech (Table 4b) as well as PRE and KRE in oak (Table 4c).

*4.3 Effects of $O_3$ and soil on foliar contents and retranslocation rates in in beech species*

According to the research reported by Conti and Geiger (1982), K is able to regulate the photosynthates translocation in sap of plants and higher K may accelerate the functions in photosynthesis process. Therefore, the decreased foliar K at amb-S and $eO_3$-S (Table 3b) were likely because of the reduced photosynthesis process caused by K-limited S soil (Table 1) and $eO_3$, respectively since both soil and $eO_3$ were found to significantly affect the foliar K content in beech species (Table 2b). Another possible explanation for this that was supported by Kayama and Koike (2015) was that the uptake K in beech leaves might be suppressed by accumulation of Mg in leaves under infertility soil as it decreases the leaf water potential and photosynthetic rate. Serpentine soil contained higher Cr and Ni (Table 1) but we did not detect any effects on Cr or Ni in beech species (Table 2b). As Al is a highly rhizo-toxic and a major stress element to plants on acid soils like V soil (Table 1) as found by Brunner and Sperisen (2013), we considered that the significant decreased content of foliar Al in beech for both infertile soil and $eO_3$ (Table 2b, 3b) likely suggested that beech has a strong detoxification mechanism (Kayama and Koike, 2015; Kayama et al., 2011) to protect itself from adverse environment condition (e.g. S soil, $eO_3$) and make the leaves suppress the Al uptake since low content of Al has beneficial effects on growth (Marschner, 2012).

With the respect of retranslocation in beech species (Table 2b, 3b), we considered that $eO_3$ may have a detrimental effect on Mg dynamics and make Mg accumulated in aged leaves to get a lower MgRE at $eO_3$. What's more, the decreased results of MgRE and CaRE in beech were likely due to the hormone changed regulated by K dynamics as KRE was found to be enhanced at EB. On the other hand, the reduction of Mg and Ca in foliar mature leaves of beech species that likely induced the lower values of MgRE and CaRE maybe due to the foliar leaching via cation exchange

(Helmisaari, 1992). Leaching of Ca in mature leaves was probably weaken at S soil due to its limited content of soil Ca (Table 1) but was likely strengthen at lower pH soil (V soil, Table 1) as reported by Gjengedal (1996) or at $eO_3$. Similarly, PRE was significantly decreased by $eO_3$ and the interaction $O_3$ x Soil (Table 4b) which was likely due to the leaching of P in senescing leaves under $eO_3$ since late leaves of birch (senescing leaves in this study) were more susceptible to $eO_3$ (Helmisaari and Mälkönen, 1989; Hoshika et al., 2013).

*4.4 Effects of $O_3$ and soil on foliar contents and retranslocation rates in oak species*

With regard to oak species combined with the results in Table 2c and 3c, oak is perhaps more tolerant than other 2 species due to fewer elements were observed to be affected by $O_3$ and/or Soil. Apparently, foliar Ca content was affected by interaction $O_3$ x Soil due to the declined Ca was identified at ES. As serpentine soil contains higher toxic elements including high ratio of Mg/Ca and excessive Ni and Cr (Table 1), in addition to the detrimental effects of $eO_3$ on the detoxification mechanism for oak species, the reduced Ca possibly suggested that the accumulation of toxic elements (Mg, Ni and Cr) in soil decreased the photosynthetic rate at $eO_3$ and thereby decrease the foliar uptake of Ca. Alternatively, there was no adequately Ca available in S soil (Table 1) to be absorbed from roots and eventually lead a lower leaf Ca in oak species. Moreover, Ca as the relatively immobile elements, dramatically reduction of CaRE may be attributed to the higher accumulation in senescing leaves that induced by the effect of infertile soil. Similar explanation is able to apply on AlRE and CrRE as well in oak species (Table 4c) for their reductions at $eO_3$ and S soil, respectively. As one of the essential components of key molecules such as nucleic acid and ATP, P is involved in regulation of enzyme reactions, promotes metabolic process and helps strengthen plants (Lawniczak, 2011).

According to the study of Thomas et al. (2006), the reduction of leaf P in oak species (Table 3c) likely depends on the soil types and on the impact degree of metabolism of $O_3$ since $eO_3$ also alters the mycorrhization as reported by Edwards and Kelly in 1992. Furthermore, PRE was found to be affected by interaction $O_3$ x Soil and it was significantly higher in both S soil and at $eO_3$ (Table 4c), suggesting that alive leaves may be able to avoid the detrimental effects of $eO_3$ and P as a mobile element is retranslocated more in living leaves (Hoshika et al., 2013). Furthermore, the lower retention of Mn and P in senescing leaves may result in the significant effects of $eO_3$ on MnRE and PRE since nutrient contents in late leaves were susceptible to be decreased by $O_3$ stress (Hoshika et al., 2013). Although KRE was found to be affected by interaction $O_3$ x soil as well (Table 2c), there was no difference between ambient and $eO_3$, suggesting that the effects of $O_3$ on foliar K dynamics is considering too small due to more effects of $eO_3$ might be detected on roots.

*4.5 Comparisons among different species*

Regarding with the compositive results of nutrient re-translocations in Table 2c and 4c, although oak is considered as the most tolerant species to $eO_3$ among the three species on the foliar contents (Kohno et al., 2005; Yamaguchi et al., 2011), it is possibly susceptible to $eO_3$ on dynamics of immobile elements (e.g. Mn, Al) and it is possible also the most sensitive to soil conditions on foliar retranslocation rates compared to other 2 species. Taking account with growing seasons for different species, we divided the discussion of results in Table 5 into two aspects. As re-translocated nutrients are available for reusing by the plant in the next year's growth, for long term monitoring on tree growth, Mg and P are retranslocated from old to younger leaves the most in beech species,

meanwhile N are translocated the most and Ni is remained considerably in oak leaves; for short-term monitoring (one growing season or less) on oak growth, foliar retranslocation of K and Mn are observed to be the most sensitive pair of elements; retranslocation of Ca, Fe, Al and N is able to be considered as the indices in monitoring the growth of birch species for either short- or long-term.

*4.6 Foliar elements contents and the elemental relationships in different species*

Generally, foliar elements contents and their retranslocation rates are varied across the species with different shoot growth patterns (Shi et al., 2016a) and mineral accumulation pattern for metal elements (Watanabe et al., 2007). Therefore, the difference foliar contents for Fe and Mn between birch and other two species, is probably related to the shoot growth and also accumulation patterns. Based on the results of varietal difference contributed to variation of foliar elements and the descriptive statistics among species shown in Table 6, considerable differences among species were also detected in all essential elements excluding Ni in leaves. Ni is generally accumulated in roots but not leaves (Jones and Hutchinson, 1988). This trend was agreed with this result as shown in Figure 2. The differences for the projected elements corresponding to variations in addition to the species were likely due to the environmental factors such as soil and climate, or the heterogeneous distribution of non-essential elements in the field (Chu et al., 2015). Although Ni is known as the metal component required for numbers of enzyme activity in biological process (Marschner, 2012), while the metabolic activity involving Cr in plants remains unclear, similar results of Cr and Ni with the previous study (Chu et al., 2015) were observed. Cr and Ni had the largest two CV values among the given elements for all the species (Figure 3) which also opposite to their lowest values on foliar concentration (Figure 2), suggesting that Cr and Ni may become the most two sensitive of the projected species grown in the field.

With regard to relationships of foliar elements, N was detected to be one of the elements pair that had negative correlations independent with the species (Figure 4). Previous evidence shows that uptake of N was suppressed by the accumulation of the several mineral toxic elements such as Mg, Ni and Cr (Kayama et al., 2005; Singh et al., 2013). We can suggest the similar trends for present species with the negative correlations of N-Fe, N-Mn and N-Ca in birch, N-Al in beech and N-Fe in oak. This trend could be attributed to the fact that accumulation of these mineral elements induces the decrease of leaf water potential, resulting in closed stomata and reduced photosynthetic rate (Rao et al., 1987; Kayama and Koike, 2015). On the other hand, Figure 5 indicated the opposite results with the positive correlations of N-K and N-Al in birch as well as N-Al in oak. Thus, interrelations between foliar N and non-essential toxic element such as Al varied with different species as summarized by Watanabe et al. (2007). For those species that positive interrelations with N-Al, the negative correlations of N-Fe were accompanied with (Figure 4a, 4b). An explanation could be that the toxic elements such as Al may have a trait that makes N uptake suppressed by those mineral elements including at least Fe (Schmitt et al., 2016). Alternatively, N uptake was suppressed by the interaction effects between Fe and other mineral elements.

Besides, birch and beech are relatively more sensitive to $O_3$ compared with oak may concern with the plenty of interactions among foliar nutrients. Especially, the uptake of N was much easier suppressed by various mineral elements when it correlated with foliar K, which closely related with the regulation of hormone control in a plant (Conti and Geiger, 1982). Nevertheless, foliar N was independent with Mg, Ni, Cr and P in studied species, which was consistent with the results of Kayama and Koike (2015). The independence between N and Mg supports our previous research

into N-Mg as a pair of major indices in assessing the $O_3$ effects in a free air system (Shi et al., 2016b).

Other correlations among mineral elements detected in this study were all positive. We found the most positive correlations in Cr and Ni, which were comparable to our previous results in Figure 2 and 3. Therefore, the sensitive of Cr and Ni to the projected species are like to be related to their highly positive interrelations with other mineral elements. Moreover, Mg, Ni, Cr as well as Mn, Fe and Ni were positively correlated with each other in all 3 datasets. As Mg, Fe and Mn assist in the chlorophyll manufacture and other biochemical process (Marschner, 2012) as well as the essential roles of Cr and Ni binding the cell wall components (Seregin and Kozhevnikova, 2006), Mg, Fe, Mn, Cr and Ni are highly involved in the basic structures and cells function, thus have similar biochemical pathways along with positive correlations (Wu et al., 2012). Furthermore, pairs of foliar elements Fe-Ni, Fe-Al and Fe-Cr have been found to highly significant correlated with $r > 0.8$ in all species, suggesting a possible link of those metal elements on their transport pathway (Schmitt et al., 2016) and the uptake of toxic mineral elements like Fe in leaves was more readily affected by other toxic mineral elements like Ni, Al and Cr.

*4.7 Profiling the foliar elements in birch, beech and oak*

Combined with the results of effects of $O_3$ and soil on foliar element content in Table 3 as well as the PCA analysis in Figure 5, the following 4 facts were observed in this study: 1) foliar Al was reduced by combined effects of $O_3$ and soil in beech as well as was notably affected by $O_3$ in oak species; 2) foliar Mn was reduced by $eO_3$ in birch as well as was markedly affected by combined effects in beech species; 3) foliar K was reduced by soil condition in birch as well as was affected by both independent treatments of $O_3$ and soil in beech; 4) Mn and Al had highest loading on PC1 as well as K had highest loading on PC2. Consequently, K and Mn, Al simultaneously responds to the treatments. But in this study, we will not take Al into account for the index elements as Al is a non-essential element and high content is toxic to plants, except some tropical species (Watanabe et al., 2007; Schmitt et al., 2016). Accordingly, when leaf Mn is reduced, $eO_3$ effect is taken into consideration, whereas when K is reduced, soil conditions are possibly the dominant effect. Therefore, Mn and K may be considered as the indices in assessing the $O_3$ and soil effects for long-term growth monitoring in seedlings of birch, beech and oak.

## 5. Conclusions

In conclusion, our research has been focused on interpreting the environmental impact factors by studying the stoichiometry of foliar nutrients in 3 broad-leaved forest species that are representative of cool temperate region of Japan. Our results suggest that both $O_3$ and soil properties are the essential impact factors on foliar stoichiometry on birch, oak, and beech seedlings.

With regard to the 3 species, oak can be considered as the most susceptible species to $eO_3$ upon leaf dynamics of immobile elements even though it was regarded as relative more tolerant species to $eO_3$ in many previous researches (e.g. Kohno et al., 2005; Yamaguchi et al., 2011). Beech with determinate shoot growth pattern is more sensitive than birch and oak species to $eO_3$ effects on foliar contents. Birch has negative correlations between foliar N and those metal elements (Ca, Mn and Fe).

Regardless the tree species, soil nutrients (soil types) have distinct impacts on retranslocation rate of K, Fe, and P. Although Cr and Ni may be the most two sensitive elements for the projected species grown on the field, the sensitivity may concern with their considerable positive interrelations

in leaf, thus they will not be the case after effects of elevated $O_3$ or/and soil conditions are taken into account. From our results, Mn and K can become indices in assessing the $O_3$ and soil effects in both short and long term monitoring on growth of these tree species.

These findings are essential in identifying the pattern of variation in leaf nutrient and its regulation variables as well as further understanding the mechanism of responses of $O_3$ tolerance in 3 species in terms of nutrient dynamics.

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Table 1. Chemical properties of brown forest soil, volcanic ash soil and serpentine soil, sampled at depth 0–10 cm (mean ± SD, n = 3–6). Pool is the main effects of the soil type, which indicates the pooled values for both ambient and elevated $O_3$ treatments.

| | Brown forest | | | Volcanic ash | | | Serpentine | | | Statistics | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amb | $O_3$ | Pool | Amb | $O_3$ | Pool | Amb | $O_3$ | Pool | Soil | $O_3$ | Soil x $O_3$ |
| pH | 5.24 | 5.30 | $5.27^{B}$ | 4.28 | 4.57 | $4.43^{B}$ | 5.90 | 6.36 | $6.13^{A}$ | *** | ns | ns |
| | (0.05) | (0.03) | (0.03) | (0.15) | (0.08) | (0.10) | (0.14) | (0.05) | (0.12) | | | |
| Mg | 16.79 | 21.97 | $19.38^{B}$ | 13.66 | 18.82 | $16.24^{B}$ | 61.76 | 75.44 | $68.60^{A}$ | *** | ns | ns |
| mg $100g^{-1}$ | (1.78) | (1.12) | (1.49) | (0.98) | (3.32) | (1.93) | (10.42) | (7.42) | (6.50) | | | |
| K | 21.25 | 26.85 | $24.05^{A}$ | 21.55 | 26.27 | $23.91^{A}$ | 12.87 | 18.98 | $15.93^{B}$ | * | ns | ns |
| mg $100g^{-1}$ | (3.66) | (3.21) | (2.51) | (1.11) | (4.12) | (2.18) | (3.61) | (2.43) | (2.38) | | | |
| Ca | 143.7 | 260.3 | $202.0^{A}$ | 116.9 | 230.4 | $173.7^{A}$ | 69.61 | 143.6 | $106.6^{B}$ | * | ns | ns |
| mg $100g^{-1}$ | (13.7) | (32.1) | (30.4) | (10.2) | (45.5) | (32.8) | (8.93) | (27.3) | (20.9) | | | |
| Mn | 1.043 | 1.081 | $1.062^{A}$ | 1.061 | 1.004 | $1.032^{A}$ | 0.736 | 0.809 | $0.773^{B}$ | * | ns | ns |
| mg $100g^{-1}$ | (0.02) | (0.16) | (0.07) | (0.02) | (0.02) | (0.02) | (0.11) | (0.09) | (0.06) | | | |
| Fe | 0.654 | 0.445 | $0.549^{AB}$ | 0.597 | 1.221 | $0.909^{A}$ | 0.266 | 0.357 | $0.311^{B}$ | * | ns | ns |
| mg $100g^{-1}$ | (0.15) | (0.07) | (0.09) | (0.12) | (0.40) | (0.23) | (0.05) | (0.12) | (0.06) | | | |
| Ni | 0.182 | 0.172 | $0.177^{B}$ | 0.172 | 0.183 | $0.178^{B}$ | 0.328 | 0.322 | $0.325^{A}$ | *** | ns | ns |
| mg $100g^{-1}$ | (0.010) | (0.008) | (0.006) | (0.006) | (0.004) | (0.004) | (0.035) | (0.029) | (0.021) | | | |
| Cr | 0.098 | 0.096 | $0.097^{B}$ | 0.097 | 0.109 | $0.103^{AB}$ | 0.115 | 0.110 | $0.113^{A}$ | * | ns | ns |
| mg $100g^{-1}$ | (0.003) | (0.004) | (0.002) | (0.004) | (0.004) | (0.004) | (0.007) | (0.004) | (0.004) | | | |
| Al | 1.303 | 0.596 | $0.950^{AB}$ | 1.108 | 1.375 | $1.242^{A}$ | 0.606 | 0.376 | $0.491^{B}$ | * | ns | ns |
| mg $100g^{-1}$ | (0.25) | (0.27) | (0.23) | (0.19) | (0.50) | (0.25) | (0.19) | (0.11) | (0.11) | | | |
| P | 2.070 | 2.317 | $2.194^{A}$ | 1.95 | 1.702 | $1.828^{A}$ | 1.924 | 2.395 | $2.160^{A}$ | ns | ns | ns |
| mg $100g^{-1}$ | (0.10) | (0.23) | (0.12) | (0.14) | (0.05) | (0.09) | (0.23) | (0.11) | (0.15) | | | |
| N | 2.308 | 2.602 | $2.455^{A}$ | 2.285 | 2.349 | $2.317^{A}$ | 1.717 | 1.332 | $1.524^{B}$ | ** | ns | ns |
| mg/g | (0.21) | (0.27) | (0.17) | (0.06) | (0.12) | (0.06) | (0.31) | (0.20) | (0.18) | | | |

ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significant.

Table 2. Levels of a two-way repeated-measures ANOVA, with collection time (Time) or growing season (GS) as within-subjects variable, for the effects of $O_3$ (ambient and elevated $O_3$ concentration) and Soil (brown forest soil, volcanic ash soil and serpentine soil) on foliar concentration of 10 elements (Mg, K, Ca, Mn, Fe, Ni, Al, Cr, P and N) and their retranslocation rates (RE) in (a) birch, (b) beech and (c) oak seedlings for 5 collections over two growing seasons.

| (a) Birch | $O_3$ | Soil | $O_3$ x Soil | Time | Time x $O_3$ | Time x Soil | Time x $O_3$ x Soil |
|---|---|---|---|---|---|---|---|
| df | 2 | 3 | 6 | 5 | 10 | 15 | 30 |
| Mg | **0.016** | 0.195 | 0.400 | **0.002** | **0.031** | 0.408 | 0.405 |
| K | 0.450 | **0.006** | 0.094 | **<0.001** | 0.341 | 0.078 | 0.219 |
| Ca | 0.494 | 0.183 | 0.445 | **<0.001** | 0.237 | 0.539 | 0.975 |
| Mn | **0.021** | 0.244 | 0.29 | **<0.001** | 0.628 | 0.625 | 0.146 |
| Fe | 0.184 | 0.077 | 0.296 | **<0.001** | **0.023** | **0.019** | 0.183 |
| Ni | 0.063 | 0.174 | 0.170 | **<0.001** | 0.847 | 0.325 | 0.08 |
| Al | 0.101 | 0.239 | 0.221 | **<0.001** | 0.157 | **0.017** | 0.196 |
| Cr | 0.284 | 0.696 | 0.444 | **<0.001** | 0.221 | 0.932 | 0.355 |
| P | **0.042** | 0.208 | 0.653 | **0.039** | 0.206 | **0.043** | 0.528 |
| N | 0.201 | 0.911 | 0.945 | **<0.001** | 0.057 | 0.122 | 0.421 |
| | $O_3$ | Soil | $O_3$ x Soil | GS | GS x $O_3$ | GS x Soil | GS x $O_3$ x Soil |
| df | 2 | 3 | 6 | 2 | 4 | 6 | 12 |
| MgRE | 0.350 | 0.805 | 0.247 | 0.889 | **0.037** | 0.973 | 0.367 |
| KRE | 0.260 | 0.276 | **0.03** | 0.056 | 0.494 | 0.257 | 0.495 |
| CaRE | 0.623 | 0.700 | 0.773 | 0.251 | 0.240 | 0.400 | 0.709 |
| MnRE | 0.838 | 0.978 | 0.422 | 0.18 | 0.372 | 0.776 | 0.124 |
| FeRE | 0.618 | 0.162 | 0.979 | 0.069 | **0.016** | 0.055 | 0.763 |
| NiRE | 0.201 | 0.535 | 0.487 | 0.174 | 0.449 | 0.940 | 0.869 |
| AlRE | 0.527 | 0.772 | 0.404 | 0.134 | 0.606 | 0.609 | 0.424 |
| CrRE | 0.470 | 0.122 | 0.194 | 0.403 | 0.502 | 0.816 | 0.278 |
| PRE | **0.004** | 0.068 | 0.991 | 0.194 | 0.244 | **0.036** | 0.49 |
| NRE | 0.454 | 0.758 | 0.086 | 0.281 | 0.084 | 0.144 | 0.058 |

| (b) Beech | $O_3$ | Soil | $O_3$ x Soil | Time | Time x $O_3$ | Time x Soil | Time x $O_3$ x Soil |
|---|---|---|---|---|---|---|---|
| df | 2 | 3 | 6 | 5 | 10 | 15 | 30 |
| Mg | 0.344 | **0.038** | 0.609 | **<0.001** | **0.032** | 0.047 | 0.29 |
| K | **0.042** | **0.037** | 0.261 | **0.007** | 0.14 | 0.13 | 0.297 |
| Ca | 0.940 | 0.051 | 0.653 | **0.001** | 0.068 | **0.009** | 0.59 |
| Mn | 0.462 | 0.053 | **0.012** | **0.002** | **0.021** | 0.177 | 0.207 |
| Fe | 0.752 | 0.056 | 0.132 | **<0.001** | **0.008** | **0.01** | 0.277 |
| Ni | 0.570 | 0.614 | 0.868 | **<0.001** | 0.232 | 0.803 | 0.435 |
| Al | **0.045** | **0.047** | **0.005** | **0.004** | 0.197 | 0.352 | 0.115 |
| Cr | 0.074 | 0.393 | 0.980 | **<0.001** | **0.016** | 0.278 | 0.525 |
| P | 0.255 | 0.148 | 0.379 | **<0.001** | 0.218 | 0.497 | 0.26 |
| N | 0.679 | 0.526 | 0.186 | **<0.001** | 0.224 | 0.054 | 0.249 |
| | $O_3$ | Soil | $O_3$ x Soil | GS | GS x $O_3$ | GS x Soil | GS x $O_3$ x Soil |
| df | 2 | 3 | 6 | 2 | 4 | 6 | 12 |
| MgRE | **0.030** | 0.340 | 0.574 | 0.105 | 0.056 | 0.198 | 0.068 |
| KRE | 0.503 | **0.030** | **0.049** | 0.037 | 0.128 | 0.229 | 0.306 |
| CaRE | **0.018** | **0.027** | 0.591 | 0.145 | 0.492 | 0.587 | 0.065 |
| MnRE | 0.169 | 0.079 | 0.225 | 0.203 | 0.175 | 0.05 | 0.057 |
| FeRE | 0.057 | 0.052 | 0.235 | 0.134 | **0.029** | **0.011** | 0.073 |
| NiRE | 0.091 | 0.700 | 0.857 | 0.267 | 0.084 | 0.558 | 0.274 |
| AlRE | 0.989 | 0.672 | 0.997 | 0.252 | 0.166 | 0.507 | 0.797 |
| CrRE | 0.136 | 0.791 | 0.370 | 0.206 | 0.318 | 0.432 | 0.933 |
| PRE | **0.012** | **0.023** | **0.018** | 0.12 | 0.921 | 0.76 | 0.133 |
| NRE | 0.203 | 0.338 | 0.571 | 0.052 | 0.078 | 0.31 | 0.058 |

| (c) Oak | $O_3$ | Soil | $O_3$ x Soil | Time | Time x $O_3$ | Time x Soil | Time x $O_3$ x Soil |
|---|---|---|---|---|---|---|---|
| df | 2 | 3 | 6 | 5 | 10 | 15 | 30 |
| Mg | 0.495 | 0.349 | 0.358 | **0.016** | 0.338 | 0.065 | 0.305 |
| K | 0.339 | 0.854 | 0.84 | **0.001** | **0.025** | 0.216 | 0.787 |
| Ca | 0.194 | 0.361 | **0.01** | 0.128 | 0.158 | 0.359 | 0.147 |
| Mn | 0.087 | 0.528 | 0.273 | **<0.001** | **0.002** | **0.001** | 0.092 |
| Fe | 0.234 | 0.062 | 0.867 | **0.001** | **0.025** | 0.176 | 0.051 |
| Ni | 0.341 | 0.771 | 0.094 | **<0.001** | 0.081 | 0.218 | 0.232 |
| Al | **0.014** | 0.534 | 0.933 | **<0.001** | 0.171 | 0.606 | 0.241 |
| Cr | 0.951 | 0.389 | 0.897 | **<0.001** | 0.974 | **0.001** | 0.91 |
| P | **0.031** | 0.419 | 0.501 | 0.329 | 0.709 | 0.158 | 0.661 |
| N | 0.802 | 0.165 | 0.356 | **<0.001** | **0.006** | 0.185 | 0.969 |
| | $O_3$ | Soil | $O_3$ x Soil | GS | GS x $O_3$ | GS x Soil | GS x $O_3$ x Soil |
| df | 2 | 3 | 6 | 2 | 4 | 6 | 12 |
| MgRE | 0.225 | 0.055 | 0.559 | 0.797 | 0.819 | 0.72 | 0.117 |
| KRE | 0.674 | **0.049** | **0.031** | 0.072 | 0.989 | 0.827 | 0.970 |
| CaRE | 0.728 | **0.014** | 0.283 | 0.112 | 0.329 | 0.073 | 0.455 |
| MnRE | **0.031** | 0.533 | 0.094 | 0.098 | 0.087 | 0.212 | 0.533 |
| FeRE | 0.199 | 0.465 | 0.297 | 0.052 | 0.416 | 0.232 | 0.165 |
| NiRE | 0.376 | 0.431 | 0.822 | 0.35 | 0.445 | 0.347 | 0.626 |
| AlRE | **0.022** | 0.198 | 0.201 | 0.173 | 0.232 | 0.139 | 0.078 |
| CrRE | 0.363 | **0.048** | 0.836 | 0.168 | 0.23 | 0.579 | 0.325 |
| PRE | **0.026** | **0.048** | **0.03** | 0.148 | 0.167 | 0.415 | 0.093 |
| NRE | 0.836 | 0.422 | 0.342 | 0.308 | 0.846 | 0.267 | 0.453 |

Table 3. Mean (±SD) foliar element concentration (mg $g^{-1}$) of Mg, K, Ca, Mn, Fe, Ni, Al, Cr, P and N in (a) birch, (b) beech and (c) oak seedlings grown on three soil types under two gas treatments. Gas treatment includes ambient $O_3$ (A) and elevated $O_3$ (E); Soil type includes brown forest soil (B), volcanic ash soil (V) and serpentine soil (S). Comparisons were made within each line among the 6 treatments. The results of each variable obtained by general linear model (GLM) analysis or a post hoc Tukey's hsd test, after significant results of the GLM analysis based on standardized data. Different letters within each row indicate statistical differences at $P < 0.05$.

| **(a) Birch** | **AB** | **AV** | **AS** | **EB** | **EV** | **ES** |
|---|---|---|---|---|---|---|
| Mg | 3.976 **b** (0.615) | 4.082 **ab** (0.585) | 5.143 **a** (1.205) | 3.643 **b** (1.186) | 3.715 **b** (0.659) | 3.742 **b** (0.788) |
| K | 8.801 **ab** (1.064) | 10.000 **ab** (2.258) | 7.962 **b** (1.541) | 10.110 **a** (1.074) | 9.294 **ab** (1.296) | 8.289 **ab** (1.360) |
| Ca | 11.630 a (1.655) | 10.440 a (1.601) | 9.999 a (1.802) | 11.507 a (1.964) | 11.632 a (1.502) | 10.890 a (1.360) |
| Mn | 0.393 **a** (0.074) | 0.373 **a** (0.068) | 0.284 **a** (0.069) | 0.219 **b** (0.044) | 0.217 **b** (0.064) | 0.215 **b** (0.051) |
| Fe | 0.419 a (0.245) | 0.328 a (0.126) | 0.318 a (0.110) | 0.383 a (0.213) | 0.343 a (0.168) | 0.292 a (0.128) |
| Ni | 0.050 a (0.036) | 0.045 a (0.030) | 0.056 a (0.035) | 0.045 a (0.032) | 0.051 a (0.037) | 0.047 a (0.032) |
| Al | 0.139 a (0.099) | 0.148 a (0.078) | 0.095 a (0.050) | 0.089 a (0.062) | 0.072 a (0.050) | 0.078 a (0.062) |
| Cr | 0.021 a (0.015) | 0.021 a (0.015) | 0.022 a (0.016) | 0.022 a (0.016) | 0.024 a (0.017) | 0.022 a (0.015) |
| P | 3.853 **ab** (0.716) | 4.356 **a** (1.331) | 3.455 **ab** (0.721) | 3.666 **ab** (0.757) | 3.437 **ab** (0.485) | 2.989 **b** (0.482) |
| N | 27.091 a (5.825) | 26.544 a (6.362) | 27.502 a (5.838) | 29.531 a (7.387) | 29.223 a (6.720) | 29.196 a (7.377) |

| (b) Beech | AB | AV | AS | EB | EV | ES |
|---|---|---|---|---|---|---|
| Mg | 1.907 **b** (0.718) | 1.803 **b** (0.577) | 3.406 **a** (0.864) | 2.128 **b** (1.032) | 1.970 **b** (0.504) | 3.069 **ab** (0.680) |
| K | 5.663 **ab** (0.817) | 6.657 **a** (1.313) | 4.460 **b** (1.080) | 5.120 **ab** (0.982) | 5.200 **ab** (0.715) | 4.261 **b** (1.116) |
| Ca | 11.688 a (1.575) | 11.498 a (1.474) | 11.694 a (1.194) | 10.865 a (1.427) | 12.162 a (1.423) | 12.318 a (1.319) |
| Mn | 0.615 a (0.236) | 0.416 a (0.093) | 0.465 a (0.128) | 0.421 a (0.140) | 0.602 a (0.267) | 0.382 a (0.133) |
| Fe | 0.314 a (0.079) | 0.272 a (0.097) | 0.249 a (0.077) | 0.260 a (0.051) | 0.288 a (0.076) | 0.308 a (0.071) |
| Ni | 0.044 a (0.032) | 0.040 a (0.035) | 0.046 a (0.033) | 0.038 a (0.035) | 0.047 a (0.033) | 0.047 a (0.033) |
| Al | 0.215 **a** (0.062) | 0.157 **ab** (0.052) | 0.108 **b** (0.034) | 0.105 **b** (0.025) | 0.146 **ab** (0.040) | 0.119 **b** (0.032) |
| Cr | 0.021 a (0.015) | 0.017 a (0.014) | 0.020 a (0.015) | 0.020 a (0.018) | 0.025 a (0.018) | 0.023 a (0.017) |
| P | 1.647 a (0.326) | 1.700 a (0.367) | 2.398 a (2.466) | 1.210 a (0.345) | 1.463 a (0.405) | 1.423 a (0.386) |
| N | 20.090 a (2.881) | 18.220 a (3.772) | 20.008 a (3.496) | 20.194 a (4.155) | 19.897 a (4.106) | 18.468 a (4.922) |

| (c) Oak | AB | AV | AS | EB | EV | ES |
|---|---|---|---|---|---|---|
| Mg | 2.412 a (0.425) | 2.204 a (0.442) | 3.011 a (0.760) | 2.887 a (1.258) | 2.028 a (0.508) | 2.920 a (0.516) |
| K | 7.709 a (1.712) | 6.866 a (1.564) | 6.828 a (1.182) | 9.041 a (1.232) | 8.565 a (2.113) | 8.430 a (2.233) |
| Ca | 13.456 **ab** (2.020) | 15.315 **a** (2.208) | 13.817 **ab** (1.858) | 14.943 **a** (1.713) | 12.716 **ab** (1.821) | 11.505 **b** (2.017) |
| Mn | 0.471 a (0.101) | 0.439 a (0.126) | 0.507 a (0.231) | 0.320 a (0.106) | 0.322 a (0.103) | 0.351 a (0.120) |
| Fe | 0.249 a (0.040) | 0.236 a (0.055) | 0.236 a (0.063) | 0.327 a (0.140) | 0.253 a (0.060) | 0.242 a (0.074) |
| Ni | 0.046 a (0.032) | 0.052 a (0.039) | 0.059 a (0.043) | 0.067 a (0.049) | 0.039 a (0.031) | 0.040 a (0.028) |
| Al | 0.144 a (0.039) | 0.130 a (0.030) | 0.112 a (0.023) | 0.128 a (0.064) | 0.124 a (0.068) | 0.100 a (0.055) |
| Cr | 0.021 a (0.015) | 0.021 a (0.015) | 0.022 a (0.017) | 0.023 a (0.015) | 0.019 a (0.015) | 0.019 a (0.015) |
| P | 2.513 **ab** (0.637) | 2.865 **ab** (0.612) | 3.944 **a** (2.504) | 2.681 **ab** (0.512) | 1.885 **b** (0.513) | 2.377 **ab** (0.540) |
| N | 23.212 a (3.529) | 21.246 a (3.882) | 24.229 a (4.136) | 23.601 a (5.015) | 20.949 a (6.125) | 24.521 a (6.415) |

Table 4. Mean (±SD) foliar element resorption efficiency (RE) of Mg, K, Ca, Mn, Fe, Ni, Al, Cr, P and N in (a) birch, (b) beech and (c) oak seedlings grown on three soil types under two gas treatments. Gas treatment includes ambient $O_3$ (A) and elevated $O_3$ (E); Soil type includes brown forest soil (B), volcanic ash soil (V) and serpentine soil (S). Comparisons were made within each line among the 6 treatments. The results of each variable obtained by general linear model (GLM) analysis or a post hoc Tukey's hsd test, after significant results of the GLM analysis based on standardized data. Different letters within each row indicate statistical differences at $P < 0.05$

| **(a) Birch** | **AB** | **AV** | **AS** | **EB** | **EV** | **ES** |
|---|---|---|---|---|---|---|
| **MgRE** | -0.413 a | -0.441 a | -0.353 a | -0.131 a | -0.347 a | -0.314 a |
| | (0.071) | (0.081) | (0.078) | (0.103) | (0.045) | (0.048) |
| **KRE** | 0.249 **ab** | 0.417 **a** | 0.294 **ab** | 0.134 **b** | 0.209 **ab** | 0.364 **a** |
| | (0.008) | (0.032) | (0.044) | (0.025) | (0.022) | (0.030) |
| **CaRE** | -0.510 a | -0.480 a | -0.478 a | -0.673 a | -0.472 a | -0.383 a |
| | (0.057) | (0.060) | (0.085) | (0.055) | (0.055) | (0.047) |
| **MnRE** | -0.638 a | -0.450 a | -0.472 a | -0.434 a | -0.657 a | -0.561 a |
| | (0.069) | (0.039) | (0.088) | (0.044) | (0.079) | (0.041) |
| **FeRE** | -2.274 a | -1.540 a | -1.186 a | -2.179 a | -1.464 a | -1.095 a |
| | (0.200) | (0.145) | (0.085) | (0.226) | (0.091) | (0.103) |
| **NiRE** | -0.543 a | -0.136 a | -0.137 a | -0.225 a | -0.113 a | -0.002 a |
| | (0.120) | (0.041) | (0.078) | (0.081) | (0.050) | (0.056) |
| **AlRE** | -4.997 a | -12.271 a | -4.499 a | -2.488 a | -1.948 a | -7.120 a |
| | (0.123) | (3.539) | (0.822) | (0.221) | (0.079) | (2.455) |
| **CrRE** | -0.627 a | -0.680 a | -0.249 a | -0.475 a | -0.329 a | -0.205 a |
| | (0.100) | (0.080) | (0.039) | (0.138) | (0.063) | (0.078) |
| **PRE** | -0.538 **b** | -0.275 **ab** | -0.256 **ab** | -0.144 **ab** | 0.105 **a** | 0.162 **a** |
| | (0.086) | (0.075) | (0.064) | (0.064) | (0.077) | (0.061) |
| **NRE** | 0.547 a | 0.589 a | 0.517 a | 0.574 a | 0.469 a | 0.540 a |
| | (0.008) | (0.016) | (0.009) | (0.018) | (0.027) | (0.015) |

| (b) Beech | AB | AV | AS | EB | EV | ES |
|---|---|---|---|---|---|---|
| **MgRE** | 0.179 **ab** (0.162) | 0.383 **a** (0.071) | 0.296 **ab** (0.080) | -0.267 **b** (0.339) | 0.085 **ab** (0.105) | -0.118 **ab** (0.179) |
| **KRE** | 0.248 **ab** (0.093) | 0.026 **b** (0.062) | 0.358 **ab** (0.045) | 0.411 **a** (0.100) | 0.192 **ab** (0.126) | 0.271 **ab** (0.178) |
| **CaRE** | 0.168 **a** (0.073) | 0.152**ab** (0.045) | -0.012 **abc** (0.063) | -0.145 **b** (0.054) | -0.045 **bc** (0.065) | -0.219 **b** (0.080) |
| **MnRE** | 0.162 a (0.179) | 0.068 a (0.077) | -0.292 a (0.178) | -0.060 a (0.022) | -0.122 a (0.168) | -0.235 a (0.072) |
| **FeRE** | -0.157 a (0.101) | -0.729 a (0.347) | -0.467 a (0.318) | -0.193 a (0.148) | -0.404 a (0.093) | -0.160 a (0.097) |
| **NiRE** | -0.454 a (0.391) | -0.663 a (0.328) | -0.320 a (0.085) | -0.197 a (0.076) | -0.111 a (0.063) | -0.025 a (0.137) |
| **AlRE** | -0.540 a (0.340) | -0.839 a (0.191) | -0.770 a (0.437) | -0.700 a (0.486) | -0.853 a (0.293) | -0.759 a (0.298) |
| **CrRE** | -0.212 a (0.156) | -0.269 a (0.292) | -0.323 a (0.100) | -0.211 a (0.085) | 0.090 a (0.101) | -0.126 a (0.086) |
| **PRE** | -0.002 **ab** (0.128) | -0.458 **b** (0.233) | 0.401 **a** (0.172) | 0.387 **b** (0.153) | 0.205 **ab** (0.109) | 0.158 **ab** (0.173) |
| **NRE** | 0.324 a (0.035) | 0.326 a (0.047) | 0.401 a (0.046) | 0.279 a (0.060) | 0.321 a (0.054) | 0.368 a (0.064) |

| (c) Oak | AB | AV | AS | EB | EV | ES |
|---|---|---|---|---|---|---|
| **MgRE** | -0.025 a (0.110) | 0.095 a (0.087) | -0.133 a (0.179) | 0.270 a (0.117) | 0.178 a (0.198) | -0.151 a (0.125) |
| **KRE** | 0.031 **ab** (0.119) | 0.146 **ab** (0.093) | 0.224 **ab** (0.075) | 0.128 **ab** (0.090) | -0.206 **b** (0.217) | 0.270 **a** (0.157) |
| **CaRE** | 0.151 **ab** (0.107) | 0.224 **a** (0.080) | -0.171 **b** (0.172) | 0.022 **ab** (0.076) | 0.011 **ab** (0.108) | -0.028 **ab** (0.105) |
| **MnRE** | -0.444 **ab** (0.205) | -1.075 **b** (0.472) | -1.033 **b** (0.429) | -0.709 **ab** (0.362) | 0.155 **a** (0.146) | -0.498 **ab** (0.374) |
| **FeRE** | -0.194 a (0.068) | -0.264 a (0.054) | -0.429 a (0.156) | -1.086 a (0.440) | -0.362 a (0.202) | -0.849 a (0.540) |
| **NiRE** | -0.132 a (0.122) | -1.522 a (0.740) | -0.380 a (0.136v | -1.146 a (0.426) | -3.618 a (3.465) | -1.031 a (0.822) |
| **AlRE** | -0.505 **a** (0.292) | -0.636 **a** (0.176) | -1.341 **a** (0.824) | -1.827 **a** (0.403) | -3.781 **ab** (2.145) | -8.071 **b** (4.452) |
| **CrRE** | -0.304 **a** (0.178) | -0.315 **a** (0.196) | -1.194 **b** (0.522) | -0.137 **a** (0.095v | -0.098 **a** (0.065) | -0.759 **ab** (0.175) |
| **PRE** | -0.325 **c** (0.248) | -0.277 **c** (0.070) | -0.194 **bc** (0.087) | -0.479 **c** (0.189) | 0.414 **a** (0.065) | 0.191 **ab** (0.082) |
| **NRE** | 0.354 a (0.052) | 0.426 a (0.033) | 0.336 a (0.062) | 0.363 a (0.063) | 0.358 a (0.080) | 0.432 a (0.072) |

Table 5. Mean (±SD) foliar element resorption efficiency (RE) of Mg, K, Ca, Mn, Fe, Ni, Al, Cr, P and N in 3 species during the growing seasons of 2014 and 2015 (GS X Species). One-way repeated-measures ANOVA followed by Tukey's hsd test, were applied within each row of the table. Different letters indicate statistical differences at $P < 0.05$

| | 1st growing season (2014) | | | 2nd growing season (2015) | | |
|---|---|---|---|---|---|---|
| | **Birch** | **Beech** | **Oak** | **Birch** | **Beech** | **Oak** |
| **MgRE** | -0.311 **c** (0.193) | -0.112 **bc** (0.316) | -0.0280 **abc** (0.191) | -0.355 **c** (0.291) | 0.280 **a** (0.139) | 0.103 **ab** (0.219) |
| **KRE** | 0.248 **a** (0.047) | 0.130 **ab** (0.096) | -0.0579 **c** (0.131) | 0.307 **a** (0.146) | 0.362 **a** (0.182) | 0.244 **a** (0.218) |
| **CaRE** | -0.462 **b** (0.134) | -0.106 **a** (0.095) | -0.0802 **a** (0.090) | -0.536 **b** (0.255) | 0.0606 **a** (0.122) | 0.154 **a** (0.199) |
| **MnRE** | -0.625 **b** (0.168) | -0.157 **ab** (0.252) | -0.839 **c** (0.567) | -0.445 **abc** (0.236) | -0.0206 **a** (0.136) | -0.362 **ab** (0.443) |
| **FeRE** | -2.102 **b** (0.549) | -0.501 **a** (0.377) | -0.579 **ab** (0.393) | -1.144 **b** (0.413) | -0.207 **a** (0.175) | -0.431 **a** (0.446) |
| **NiRE** | -0.272 **ab** (0.194) | -0.174 **a** (0.126) | -0.425 **ab** (0.253) | -0.113 **a** (0.315) | -0.388 **ab** (0.411) | -2.210 **c** (2.938) |
| **AlRE** | -2.793 **b** (0.537) | -0.467 **a** (0.226) | -0.692 **a** (0.276) | -8.314 **b** (8.418) | -0.986 **a** (0.574) | -4.430 **a** (3.924) |
| **CrRE** | -0.241 **ab** (0.189) | -0.0654 **a** (0.093) | -0.287 **ab** (0.232) | -0.614 **b** (0.359) | -0.266 **ab** (0.269) | -0.651 **b** (0.518) |
| **PRE** | 0.0291 **ab** (0.134) | -0.110 **b** (0.186) | -0.235 **b** (0.247) | -0.345 **b** (0.325) | 0.332 **a** (0.277) | 0.0157 **ab** (0.257) |
| **NRE** | 0.576 **a** (0.023) | 0.300 **d** (0.064) | 0.291 **d** (0.045) | 0.502 **ab** (0.073) | 0.373 **bcd** (0.073) | 0.464 **c** (0.083) |

| | df | MgRE | KRE | CaRE | MnRE | FeRE | NiRE | AlRE | CrRE | PRE | NRE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GS | 2 | 0.155 | **0.009** | **0.002** | **0.013** | **0.006** | 0.131 | **0.023** | **0.002** | 0.152 | **0.035** |
| Species | 3 | **0.001** | **0.004** | **<0.001** | **0.001** | **<0.001** | 0.093 | **0.011** | 0.071 | **0.026** | **<0.001** |
| GS x Species | 6 | 0.065 | 0.208 | **0.027** | 0.299 | 0.075 | **0.034** | 0.193 | 0.653 | **<0.001** | **<0.001** |

Table 6. Species variation in foliar elements concentrations

| | species | F | mean | SD | min | max |
|---|---|---|---|---|---|---|
| Mg | birch | 16.03* | 4.0894[a] | 1.2606 | 2.3871 | 6.6165 |
| | beech | | 2.4748[b] | 1.3741 | 0.6949 | 6.1721 |
| | oak | | 2.5864[b] | 0.9942 | 1.2350 | 5.5529 |
| K | birch | 23.24* | 9.0796[a] | 2.3828 | 4.3099 | 14.1991 |
| | beech | | 5.3147[b] | 1.7223 | 1.7331 | 10.1238 |
| | oak | | 7.8517[a] | 2.3420 | 4.1736 | 13.5101 |
| Ca | birch | 9.71* | 11.0541[b] | 2.3781 | 6.2758 | 15.3645 |
| | beech | | 11.8342[b] | 1.8363 | 8.6612 | 15.6483 |
| | oak | | 13.6253[a] | 2.6271 | 9.0903 | 21.3712 |
| Mn | birch | 6.23* | 0.2877[b] | 0.1174 | 0.1098 | 0.5504 |
| | beech | | 0.5055[a] | 0.3127 | 0.2101 | 1.3769 |
| | oak | | 0.4075[a] | 0.2362 | 0.1244 | 1.2521 |
| Fe | birch | 7.78* | 0.3546[b] | 0.2943 | 0.1020 | 1.2221 |
| | beech | | 0.2938[a] | 0.1340 | 0.1662 | 0.6815 |
| | oak | | 0.2580[a] | 0.1240 | 0.1368 | 0.7508 |
| Ni | birch | 0.02 | 0.0506 | 0.0576 | 0.0018 | 0.1450 |
| | beech | | 0.0492 | 0.0580 | 0.0010 | 0.1478 |
| | oak | | 0.0520 | 0.0645 | 0.0004 | 0.2049 |
| Al | birch | 1.63 | 0.1069 | 0.1145 | 0.0104 | 0.4629 |
| | beech | | 0.1476 | 0.0791 | 0.0575 | 0.3585 |
| | oak | | 0.1221 | 0.0632 | 0.0297 | 0.2685 |
| Cr | birch | 0.04 | 0.0227 | 0.0266 | 0.0006 | 0.0628 |
| | beech | | 0.0238 | 0.0282 | 0.0007 | 0.0660 |
| | oak | | 0.0217 | 0.0262 | 0.0005 | 0.0670 |
| P | birch | 24.29* | 3.6539[a] | 1.0589 | 2.0734 | 6.0450 |
| | beech | | 1.6554[c] | 0.9256 | 0.2657 | 5.2749 |
| | oak | | 2.7467[b] | 1.2921 | 0.8342 | 7.7117 |
| N | birch | 7.56* | 27.9096[a] | 10.8585 | 13.3667 | 45.5064 |
| | beech | | 19.4458[b] | 5.8048 | 11.2000 | 31.4683 |
| | oak | | 22.9563[b] | 7.8284 | 13.0833 | 40.8718 |

Note: Pooled data of all O3 and soil treatments carried out in two years were shown in the table (n=29-30); F values generated by ANOVA with Tukey test; max (maximum), min(minmum), mean with the unit of mg $g^{-1}$; SD(standard deviation) of elemental accumulation in leaves for the elements; *, $P$<0.001; different letters in the same element's column means significant differences among species ($P$<0.05, ANOVA with Tukey test).

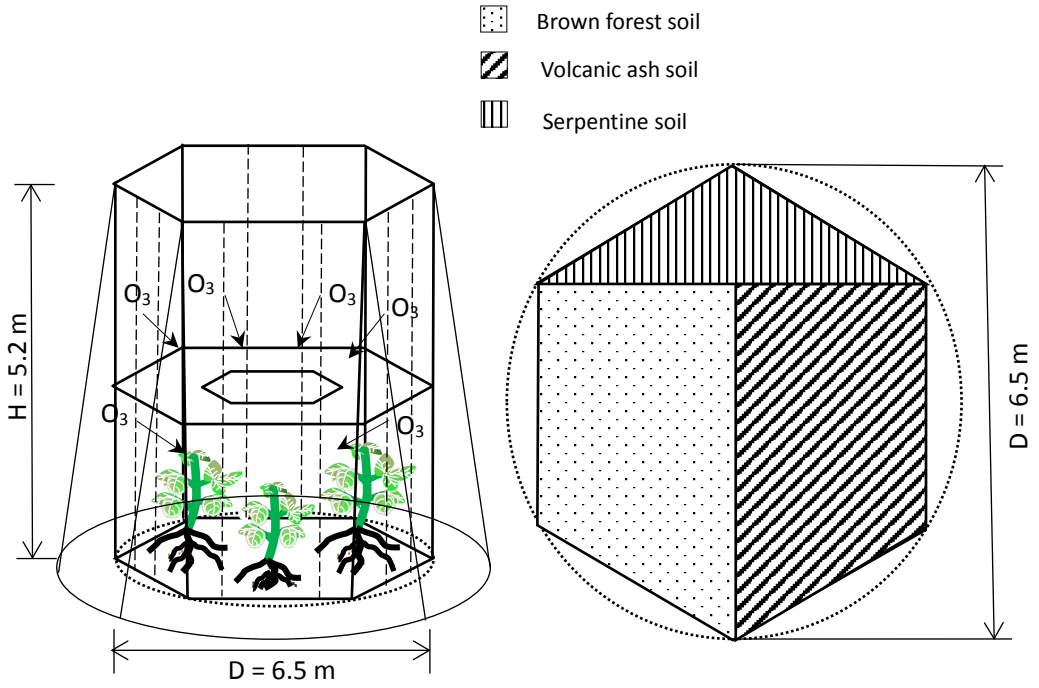


Figure 1. Design of a free-air enrichment experimental circular plot and a bird-view of soil distribution for each plot at Hokkaido University experimental nursery.

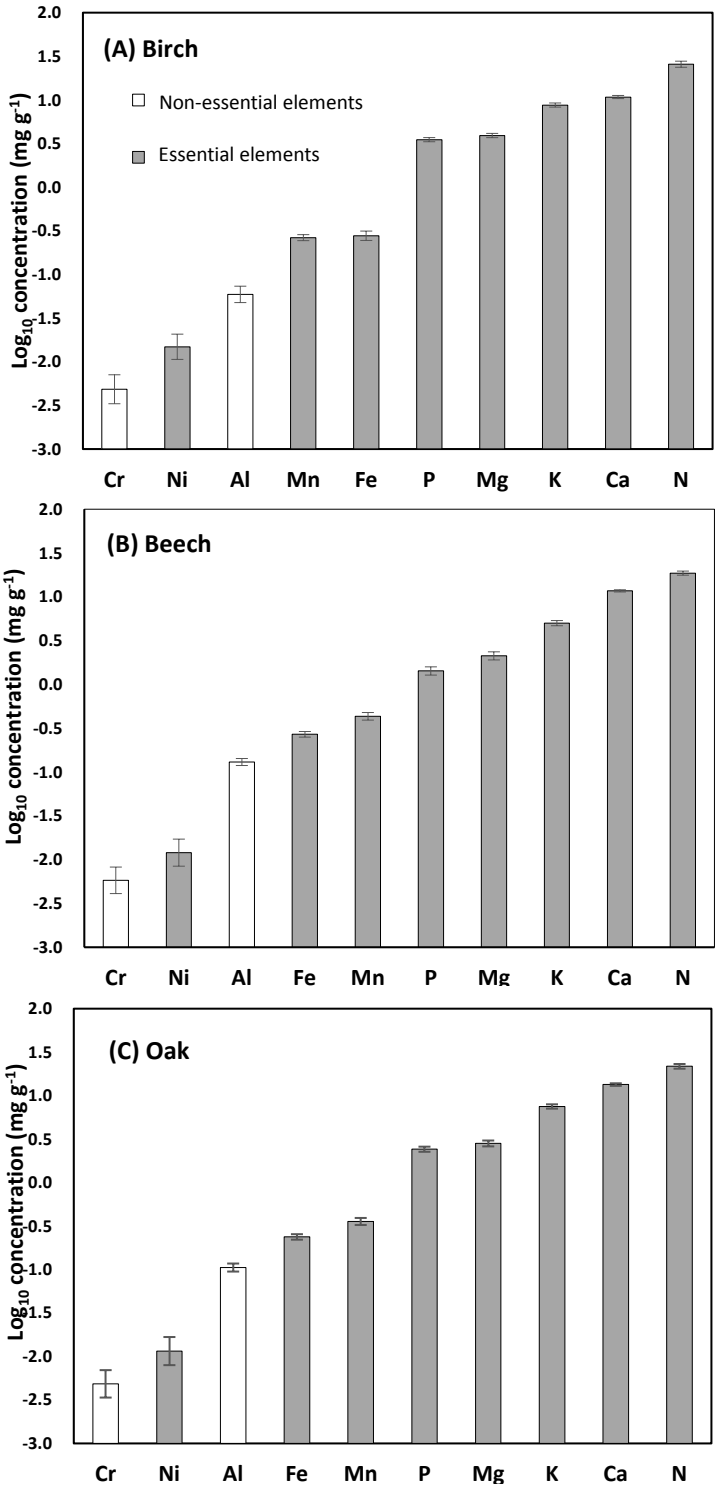


Figure 2. Average foliar contents of 10 elements in (A) Birch, (B) Beech and (C) Oak species Black columns indicate the essential elements, while white columns indicate the non-essential elements.

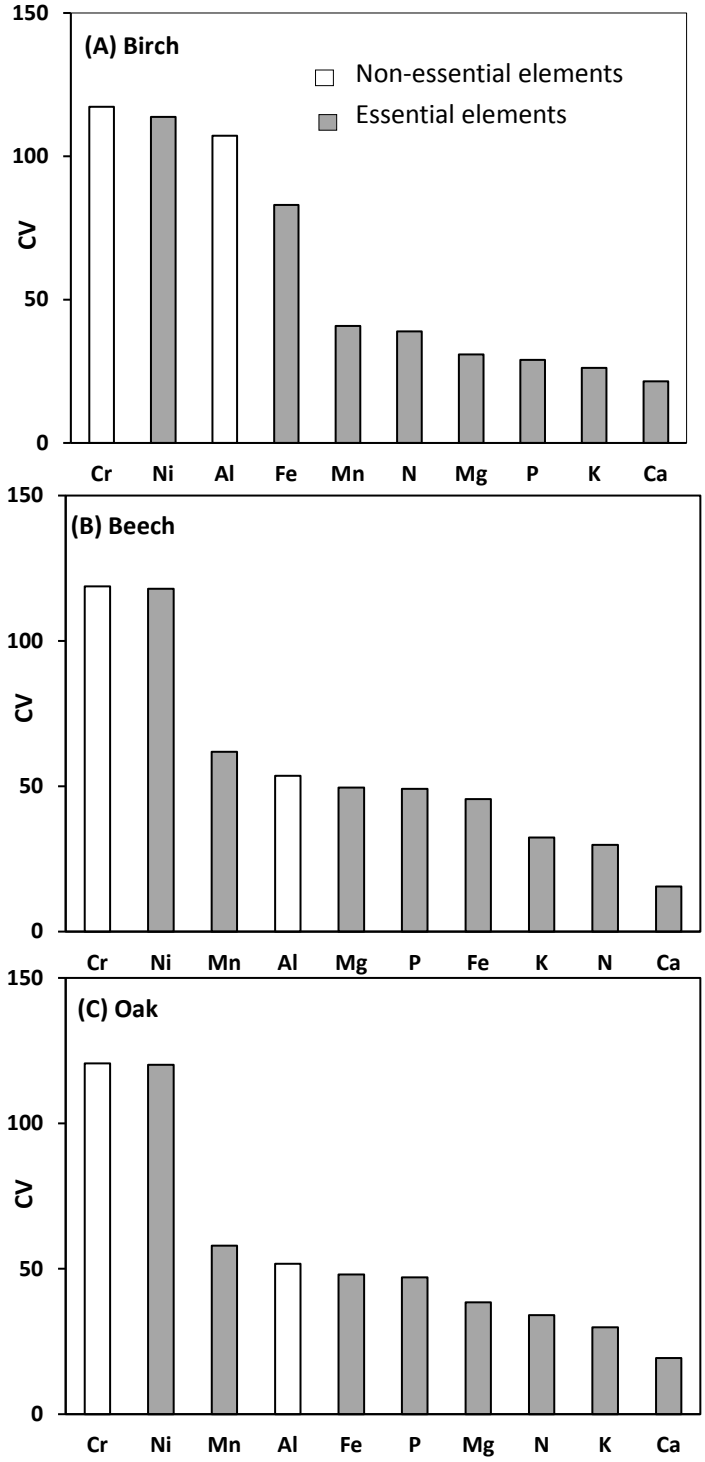


Figure 3. Relationship between average foliar contents and their coefficients of variation (CV) in (A) Birch, (B) Beech and (C) Oak. CV is the ratio of standard deviation values to average values of all seedlings for each species. Black columns indicate the essential elements, while white columns indicate the non-essential elements.

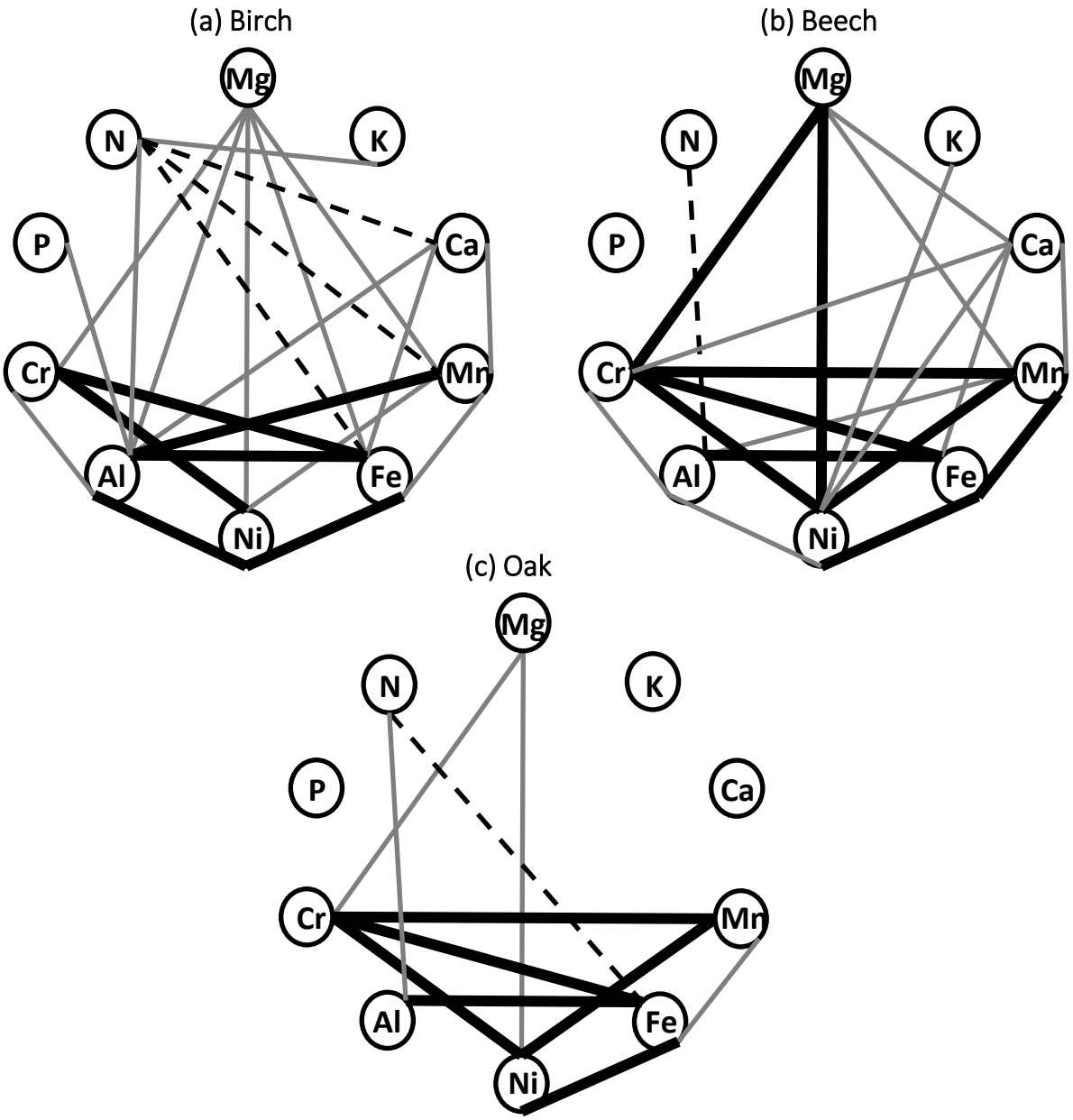


Figure 4. Correlations wheels showing significant correlations between foliar elements of (a) birch (b) beech (c) oak seedlings measured during two growing seasons. Correlations were derived from Pearson r correlation analysis and only significant correlation with $p < 0.001$ and $r > 0.5$ are illustrated on each wheel. Thin gray lines denote when $0.5 < r < 0.7$, and thick black lines denote $r \geq 0.7$. Solid lines indicate positive correlations, and dashed lines indicate negative correlations.

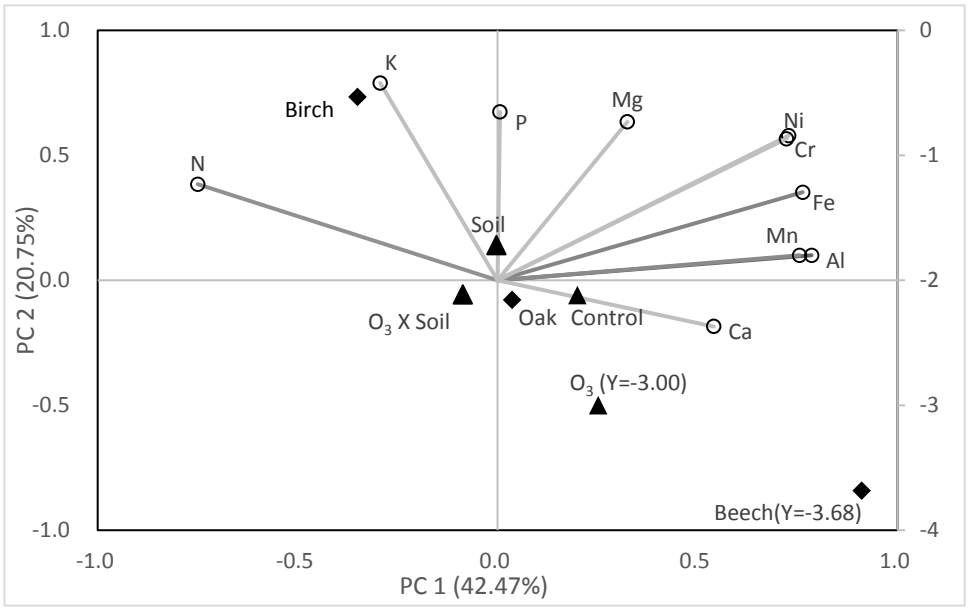


Figure 5. Biplot of the PCA results for foliar elements. “Control” indicates the Ambient $O_3$ with brown forest soil condition. Circular dot points stand for the scores of each response variable; triangle dot points stand for the scores of the treatments of $O_3$ and/or soil; rhombus dot points stand for the scores of species. (Y indicates the scores loading on axis of PC2, based on the scale in right side)