Title Physiological and stoichiometry study on foliar nutrients and defensive characteristics of representative deciduous broad-leaved tree species in northern Japan under environmental changes

Introduction

The global environment has been dramatically changing due to human activities, especially elevated atmospheric carbon dioxide (CO_2), ozone (O_3), and nitrogen (N) as a precursor for O_3. Atmospheric CO_2 has been globally increasing and promotes plant growth via photosynthetic enhancement for an extent. At the same time, ground-level O_3, as one of the serious environmental stresses for plants, has also been continuously increasing in Asia. As O_3 has strong oxidative characteristics, O_3 via stomata penetrating plants and induces morphological and chemical changes in leaves. This can accelerate foliar senescence and negatively affect the vigor and health of forests. Moreover, soil conditions (e.g. nutrient availability) also affect the physiological function of both nutrient retranslocation and plant defense.

Representatives of deciduous broad-leaved tree species native to the northern forests of Japan, such as, Japanese white birch (*Bp*: *Betula platyphylla* var. *japonica* Hara), Siebold's beech (*Fc*: *Fagus crenata* Blume), Mizunara oak (*Qm*: *Quercus mongolica* Fisch. ex Ledeb. var. *crispula* (Blume) Ohashi) and Konara oak (*Qs*: *Quercus serrata* Murray) are considered to be the essential components. Specific O_3 sensitivity have been estimated by former experiments with potted plants in open top chambers for the beech, birch and oak, which have different shoot developmental patterns as determinate, indeterminate and semi-determinate, respectively.

Therefore, my research concerning the physiological and stoichiometry study on these trees will be of help for their adaption investigation to environmental changes and will be utilized on developing appropriate strategies of afforestation in the future. To assess the responses of the above species on various environmental changes, three researches have been carried out with a free-air enrichment system to simulate forest ecosystems.

Firstly, I investigated the physiological effects of elevated CO_2 (eCO_2) and/or elevated O_3 (eO_3) on leaf nutrient status of oak species. I chose two oak (*Qm* and *Qs*) species as the Japanese representative of *Quercus* species that more tolerant to O_3. Secondly, I examined the stoichiometry of foliar element concentrations and retranslocation of three species to eO_3 under three different soils (brown forest: B, volcanic ash: V, and serpentine: S) over one or two growing seasons and I also discussed on the plausible understanding of physiological functions. Finally, I studied foliar defensive characteristics of three species in responses to eO_3 under two soil conditions (B and V) in relation to protection capacity of the species against biological stresses via eO_3.

1. Foliar nutrients chemical composition of oak species to elevated CO_2 elevated O_3

Oaks are regarded as O_3 stress tolerant species among 18 woody plants tested in Japan while *Qm* is considered to be more tolerant to O_3 compared to *Qs.* At eCO_2, stomatal conductance is low, which results in suppression of O_3 absorption via stomata. As eCO_2 may have combined effects with eO_3 on growth and photosynthetic capabilities of the two oak species grown under B soil, I investigated foliar nutrients composition (P, N, K, Mg, Mn, Ca) as well as the foliar carbohydrates (starch and sugar) amount of 2-year-old oak seedlings (*Qm* and *Qs*) exposed to eCO_2 and/or eO_3 with a free-air enrichment system. From the results of element concentration, it was found that N and Mg may have the potential to be major indicators in assessing the effects of O_3 on two oaks. I also found that *Qs* may have a higher ability of recovering from O_3 damages and likely become more tolerant to eO_3 than *Qm* under eCO_2 independent of sugar and starch concentration.

2. Foliar element concentrations, the retranslocation and seasonal changes of three species to

elevated O_3 under three different soils

This study was divided into two sections. Retranslocation is the amount of an element that is depleted from aged plant components and is provided for new growth. As leaf senescence is usually accelerated at eO_3 and leaf shedding is also influenced by soil nutrient availability (and acidification), 2 year-old (as of 2014) seedlings of *Bp*, *Fc*, and *Qm* were planted in a free-air O_3 enrichment system under 3 soil types (B, V and S).

For the first section (chapter 3), I focused on the net retranslocation of foliar nutrients (major elements: N, P, K, minor elements: Ca, Fe, Mg, Mn, and non-essential element: Al) to discuss potential effects of eO_3 on seedlings in relation to different soil conditions via retranslocation traits. I also found that the retranslocation rate of P was increased by eO_3 in *Bp* and by soil treatment in *Qm*; but constant across treatments in *Fc*. Retranslocation of N was affected by soil in *Qm*. Retranslocation of other elements was most sensitive to both eO_3 and soil in *Fc*. I could not detect molybdenum because of too small value.

For the second section (chapter 4), in addition to foliar elements studied above, I added foliar Ni and Cr as an additional minor essential and non-essential element, respectively. This was to further estimate the effects of eO_3 alone and together with different soil conditions on seasonal changes and physiological understanding of stoichiometry of the foliar elements of three species. Relationships among the foliar elements within each species were also investigated. I found that *Fc*, with a determinate shoot growth pattern, was relatively more sensitive to O_3 stress on foliar contents, meanwhile *Qm* was possibly susceptible to O_3 concerning dynamics of immobile elements. Soil nutrients had distinct impacts on retranslocation rate of K, Fe, and P. Principal component analysis revealed that Mn and K can become indicators in assessing O_3 and soil effects in both short and long term growth monitoring of these tree species.

3. Foliar defense characteristics of three species to elevated O_3 under two different soils

Plants defend themselves against herbivores by employing mainly physical and chemical defense mechanisms. As leaf defense depends on the strong influence of both genotype and environmental conditions, I investigated the leaf chemical defense traits by analyzing the C/N ratio and amount of defensive compounds (lignin, secondary metabolites production: total phenolics and condensed tannin) in response to eO_3 under B and V soils for *Bp*, *Fc* and *Qm* species as components of Fagaceae. In this study, foliar defensive traits were affected by eO_3 for *Bp*; N and C/N were influenced by soils but defensive chemicals were by eO_3 for *Fc*; *Qm* as a tolerant species, it was able to survive under various environment changes.

In conclusion, my results provide the evidences that physiological explanation on stoichiometry of foliar elements and their dynamic variation as well as foliar defensive traits are varied upon environmental changes for each species and they are also species specific. Although *Qm* is regarded as an O_3 tolerant species, after making comparisons among species, it can be susceptible to O_3 concerning dynamics of immobile elements. My findings are essential in further comprehension to nutrient ecophysiological mechanism in the nutrient dynamics of cool-temperate forests of Japan.