博 士 論 文 の 要 約

博士の専攻分野の名称： 博　士（農学）　　氏名　　Shi, Cong (石聡)

学 位 論 文 題 名

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

（変動環境における北日本の落葉広葉樹の葉成分と防御特性に関する生理的・化学量論的研究）

要約

This thesis consists of total 164 pages including 14 Figures, 17 Tables, and 6 chapters, with 3 references.

General introduction:

In chapter 1, I made a general introduction about environmental changes, tree species as well as general description about nutrients stoichiometry and defense characteristics.

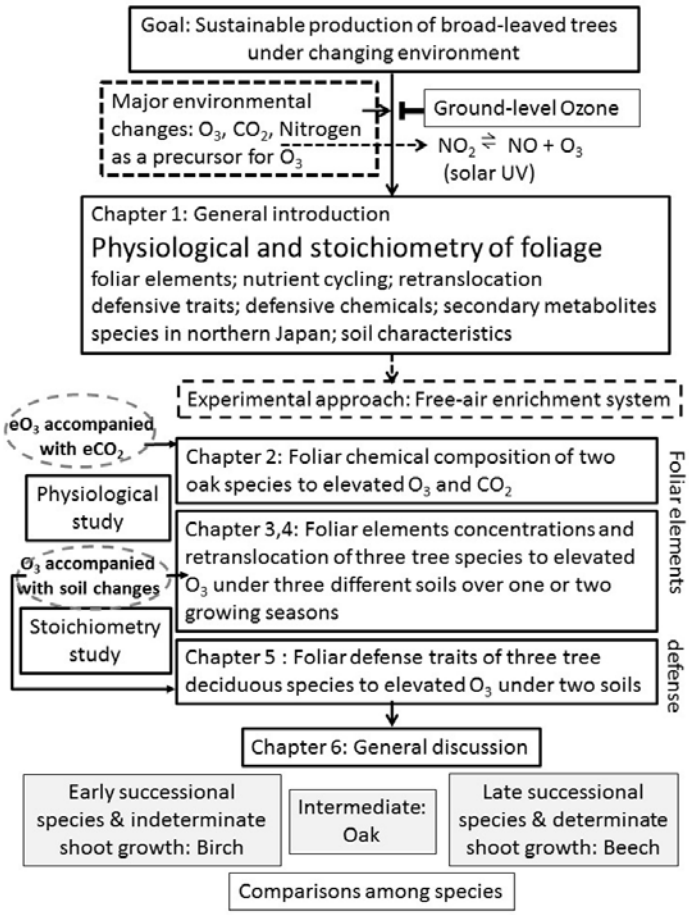

Fig. Outline of PhD thesis of C. Shi

In natural forests, uptake and release are two important factors due to representative of primary flux in ecosystem. Nutrients are absorbed from soil and being used for plants growth, physiological responses or being returned via decomposition through litter fall and being available again for next nutrient cycling. Consequently, stoichiometry is dependent on the balance among uptake, amount of retranslocation rate and loss of the processes. At the same time, plant defense is a vital mechanism for biological conservation and improvement of which is essential on forest maintenance and biological diversification.

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 has also been continuously increasing especially in Asia. Elevated O_3 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.

As representatives of deciduous broad-leaved tree species native to northern Japan, 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 applied to this study. Specific O_3 sensitivity has been estimated in sequence of beech, birch and oak, with determinate, indeterminate and semi-determinate shoot growth pattern, respectively.

To assess the responses of the above species on various environmental changes, three researches which divided into 4 chapters have been carried out with a free-air enrichment system to simulate forest ecosystems.

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

In chapter 2, I investigated foliar nutrients composition in 3 weedy plants treated with elevated O_3 (eO_3) accompanied with elevated CO_2 (eCO_2).

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, I investigated foliar nutrients composition (Ca, K, Mg, Mn, N, P) 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. The data indicated that net photosynthetic rate was decreased by eO_3 but slightly recovered by eO_3 and eCO_2 at the 1st flush and by eCO_2 at the 2nd flush for *Qm*; while it was increased by eO_3 and eCO_2 for *Qs* during the entire flush time. These trends may suggest 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. 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.

Second research concerning about foliar stoichiometry of three species to eO_3 in three different soils, was divided two chapters (chapter 3 and 4). 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 three different soil types (brown forest: B, volcanic ash: V, and serpentine: S).

【published: 公開済】→Shi, C., Kitao, M., Agathokleous, E., Watanabe, M., Tobita, H., Yazaki. K., Kitaoka, S., and Koike, T. (2016). Foliar chemical composition of two oak species grown in a free-air enricment system with elevated O_3 and CO_2. *Journal of Agricultural Meteorology*, 72:1-6. (DOI: 10.2480/agrmet.D-14-00018) (Open access journal)

2. Physiological responses on foliar nutrients chemical composition of

two oak species to elevated ozone and carbon dioxide

This part is shown as chapter 3, I focused on the net retranslocation of foliar elements (N, P, K, Ca, Fe, Mg, Mn and 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 both P and N was increased by eO_3, which could be explained from two aspects. From the view of foliar contents, it could be attributed to aged leaves are more susceptible to O_3 stress than alive leaves because younger leaves have a higher capacity of resistance against O_3 damage; From the view of foliar phenology, it is because eO_3 accelerates the foliar senescence that makes more nutrients retranslocated into younger leaves. Retranslocation of other elements was more efficient in *Fc*, which has a determinate shoot growth pattern and high sensitivity to O_3 on basis of the previous studies on classification (from sensitive to tolerant: beech > birch > oak).

【published: 公開済】→Shi,C., Eguchi, N., Meng, F, Watanabe, T., Satoh, F. and Koike. T. (2016) Retranslocation of foliar nutrients of deciduous tree seedlings in different soil condition under free-air O_3 enrichment. *iForest - Biogeosciences and Forestry* (DOI: 10.3832/ifor1889-009)
(Open access journal)

3. Stoichiometry study on various foliar elements of three deciduous tree species exposed to elevated ozone under three different soils over two growing seasons

This part is shown as chapter 4, in addition to above elements, I added foliar Ni and Cr to further estimate the effects of eO_3 alone and together with different soils over two growing seasons. I investigated on physiological understanding of stoichiometry of foliar elements as well as relationships among the foliar elements within each species. I found *Fc* with a determinate shoot growth pattern, was relatively more sensitive to O_3 stress on foliar contents, but *Qm* was possibly susceptible to eO_3 concerning dynamics of immobile elements. Moreover, I also applied principal component analysis (PCA) to profile elements in order to observe any indices elements to evaluate the O_3 and soil effects for those tree species. PCA results in my study revealed that K and Mn can become indicators in assessing O_3 and soil effects for long-term growth monitoring on *Bp*, *Fc* and *Qm*. These findings could be further proved by the independent relationship between foliar K and Mn within each species.

【published: 公開済】→Shi, C., Watanabe, T. and Koike, T (2017) Leaf stoichiometry of deciduous tree species in different soils exposed to free-air O3 enrichment over two growing seasons. Environmental and Experimental Botany.138:148–163

5. Leaf defense characteristics of deciduous tree species seedlings in different soils exposed to a free-air O_3 enrichment system

Plants defend themselves against herbivores by employing mainly chemical and physical defense mechanisms. Most plant defense are originated from photosynthates. However elevated O_3 suppresses photosynthetic capacity. Consequently plants are sensitive to insect herbivores under elevated O_3. As leaf defense depends on the strong influence of genotype of both species and environmental conditions, we investigated the leaf defense characteristics by analyzing C/N and amount of defensive compounds

(secondary metabolites production: lignin, total phenolics and condensed tannins) in response of elevated O_3 under different soils (brown forest soil and volcanic ash soil) for birch (*Betula platyphylla* var. *japonica*), beech (*Fagus crenata*) and oak (*Quercus mongolica* var. *crispula*) species. In our study, foliar defensive characteristics are affected by eO_3 in birch species; N and C/N were influenced by soils but defensive chemicals were by eO_3 in beech species; oak as an O_3-tolerant species which is competitive to be survival under various environment changes is also competitive to defense against grazing by insect herbivores.

Keywords: chemical defense, free-air ozone, volcanic ash soil, deciduous broad-leaved species

【not published yet】

6. General description of foliar nutrients and secondary metabolites under environmental changes

Last but not least, in the last chapter (chapter 6), I made a general discussion about my overall results throughout my study. Environmental changes including, eO_3, eCO_2 and various soils considerable affects dynamics of physiological processes as well as defensive characteristics of the involving species applied in my experiments. After comparisons among three species, the effects of O_3 and soils varied across species with different shoot growth patterns as well as successional characteristics. For foliar contents, beech species with determinate shoot growth pattern is more sensitive but oak is less sensitive to eO_3. For foliar dynamics of immobile elements, however, oak can be considered as relatively susceptible species to eO_3 among those tree species. After profiling elements, N-Mg are able to be indices in assessing eO_3 accompanied with eCO_2 for oaks (*Qs* and *Qm*); while K-Mn possibly become index elements to evaluate eO_3 accompanied with different soils for *Bp*, *Fc* and *Qm* species. For defense characteristics, birch shows an early-successional heterophyllous species which as a shorter lifespan within its indeterminate shoot growth is relatively susceptible to insect herbivores.

Conclusion:

My results provide 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 most of the previous researches are based on the assumption that *Qm* is an O_3 tolerant species, my study essentially provides a new evidence that *Qm* can be susceptible to O_3 as well when concerning with the dynamics of immobile elements. My findings are very important in further comprehension to nutrient ecophysiological mechanism in the nutrient dynamics of cool-temperate forests of Japan and will be of help for the investigation of species adaption to environmental changes, which will be utilized on developing appropriate strategies of afforestation in the future.