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Author(s)	KOIKE, Takayoshi; KITAOKA, Satoshi; MASYAGINA, OXANA V; WATANABE, Yoko; JI, Donghun; MARUYAMA, Yutaka; SASA, Kaichiro
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## Nitrogen Dynamics in Leaves of Deciduous Broad-leaved Tree Seedlings Grown in Summer Green Forests in Northern Japan

KOIKE Takayoshi<sup>1,2\*</sup>, KITAOKA Satoshi<sup>1</sup>, MASYAGINA Oxana V.<sup>1,3</sup>, WATANABE Yoko<sup>1</sup>, Ji Donghun<sup>4</sup>, MARUYAMA Yutaka<sup>5</sup> and SASA Kaichiro<sup>1</sup>

<sup>1</sup> Hokkaido University Forests, FSC, Sapporo 060-0809, Japan

<sup>2</sup> Department of Forest Science, Hokkaido University, Sapporo 060-8589, Japan

<sup>3</sup> V. N. Sukachev Institute of Forest, Krasnoyarsk, Russia

<sup>4</sup> Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan

<sup>5</sup> Forestry and Forest Products Research Institute, Sapporo 062-8516, Japan

### Abstract

Dynamics of nitrogen in leaves of trees and seedlings of deciduous broad-leaf species were studied for improving forest management. The photosynthetic traits of canopy leaves of deciduous broad-leaved trees were measured using a canopy tower. There was a clear positive correlation between leaf nitrogen content and light saturated photosynthetic rate at sunny canopy. As reflecting the shoot development pattern, leaf senescence began from the inner part of the crown in early successional species, by contrast leaf senescence process commences from either the outer or top portion of the crown in late successional species. For regenerated seedlings in forest floor, seasonal change in the allocation pattern of leaf nitrogen coincided with the changing of light conditions through the leaf phenology of upper canopy. In light limited condition, nitrogen was allocated to chlorophyll while it allocated to more to photosynthetic carbon fixation, i.e. Rubisco in strong light condition. Nitrogen allocation was affected more by light to CO<sub>2</sub> condition that was found in birch and beech seedlings with a FACE system.

*Key words:* canopy photosynthesis, chlorophyll and nitrogen, nitrogen allocation, elevated CO<sub>2</sub>, leaf anatomy

**Abbreviations:** Rubisco: ribulose-1,5-bisphosphate carboxylase/oxygenase, Chl: Chlorophyll, FACE: Free Air CO<sub>2</sub> Enrichment, IRGA: Infra-red Gas Analyzer, LMA: Leaf mass per area, Chl/N: the ratio of chlorophyll to nitrogen in a leaf, Psat: photosynthetic rate at light saturation at ambient CO<sub>2</sub> concentration, FACE: Free Air CO<sub>2</sub> Enrichment.

### Introduction

Nitrogen is one of the most essential elements for plant growth (e.g. Osmond 1987, Evans 1989), however, it is often a limited resource in boreal forest ecosystems (Nordin and Näsholm 1997). Therefore, we should know the dynamics of nitrogen of tree species in field for increasing biomass production and forest management. The pattern of nitrogen allocation to various organs in leaves is strongly affected by environmental factors, especially by light conditions because more than 70% of nitrogen is localized in photosynthetic organs (Hirose and Werger 1987, Evans 1989, Kitaoka and Koike 2004).

The light condition of deciduous forest floor undergoes large seasonal changes. How do the seedlings grow in understory of a deciduous forest cope with seasonal changes in the light environment, and utilize nitrogen in leaves? It is considered that tree seedlings may allocate well nitrogen to various organs in a leaf. Based on the observations of phenological pattern (Koike 2004, Kitaoka and Koike 2004), we

predicted that light-demanding (early and mid successional) species allocate more nitrogen in a leaf to Rubisco. In contrast, late successional tree species are expected to allocate more nitrogen to chlorophyll for light harvesting. Compared to seedlings grown under full sunlight, leaves of tree seedlings grown in shade should have lower leaf nitrogen content, i.e. higher Chl content, and lower Chl a/b ratio (e.g. Osmond 1987, Givnish 1988). These low light adaptations suggest a trade-off between Chl complex protein and Calvin cycle enzyme (Evans 1989, Hikosaka and Terashima 1995).

Moreover, CO<sub>2</sub> concentration [CO<sub>2</sub>] in a summer green forest changes greatly in day and season (Koike et al. 2001, 2004). Seedlings regenerated in forests understory should be exposed to such large variation of CO<sub>2</sub> environment, especially under long term high [CO<sub>2</sub>] exposure, which may induce the different allocation of leaf nitrogen to Rubisco (Karatsu et al. 2006).

To assess these predictions, we monitored seasonal changes of maximum leaf photosynthetic rates *in situ*, nitrogen, chlorophylls and Rubisco content in leaves of trees and seedlings with typical phenological patterns. We also studied on the allocation patterns of nitrogen in leaves of seedlings grown in elevated CO<sub>2</sub> environment with a FACE system.

## Materials and Methods

### 1) Study sites and Plant species

The canopy study site was located in the experimental forest of the Forestry and Forest Products Research Institute at Sapporo (42°58'N, 141°23'E). We built a 24 m monitoring tower in a mixed deciduous broad-leaved forest (about 70 years old in 2005) at a point where seven canopy tree species were accessible for physiological measurements. Tree seedlings were used in unmanaged larch stands (51 years old in 2005) at the Tomakomai Experimental Forest (42°40'N, 141°36'N). All sites have average precipitation of about 1200 mm.yr<sup>-1</sup> and temperature of 5.8~6.3°C.

Leaf photosynthesis at various canopy profiles was determined for seven species: walnut (*Juglans ailanthifolia*; mid successional species), alder (*Alnus hirsuta*; early successional), white birch (*Betula platyphylla* var. *japonica*; early successional), basswood (*Tilia japonica*; late successional), maple (*Acer mono*; late successional), elm (*Ulmus davidiana* var. *japonica*; mid successional) and ash (*Fraxinus mandshurica* var. *japonica*; mid successional; Koike et al. 1998). Other species near the tower but not measured include oak (*Quercus mongolica* var. *crispula*; mid-late successional), dogwood (*Cornus contraversa*; mid successional) and Caster aralia (*Kalopanax septemlobus*; mid successional). The mean tree height was 18.5 m and the average diameter at breast height was 1.58 m (SD 0.12m).

To study seedlings regenerating in an unmanaged larch plantation, we examined seedlings as follows: magnolia (*Magnolia hyporeuca*, mid successional), carpinus (*Carpinus cordata*, late successional), cherry (*Prunus ssiori*, late successional) and oak (mid-late successional).

In FACE, we measured both sunny and shady leaves in birch (*Betula platyphylla* var. *japonica*) and only shade leaves of beech (*Fagus crenata*, late successional) because the height of beech was relatively smaller. Thus we could only obtain shade leaves of beech. Mature third, fourth or fifth leaves counted from the shoot tip were chosen from four plants for measurement of gas exchange rates and leaf characteristics. Nomenclature is mainly followed by Ohwi (1983).

### 2) Measurement of photosynthetic capacity and leaf characteristics

The photosynthetic photon flux (PPF) at different canopy heights (0.5, 2, 4, 10, 14, 18 and 24 m) was recorded. The relative PPF (rPPF) was determined as the ratio of PPF at an open site to the PPF at a chosen place in the forest. The light saturated photosynthesis (P<sub>sat</sub>) and photosynthetic light response curve were measured using an open system portable IRGA (ADC H4, UK; LI-6400, LiCor, Lincoln, Nebraska, USA). Leaf area was determined using an area meter (LI-300, LiCor) following the photosynthesis measurements. The leaf was then dried for 24 hrs at 65°C for dry mass determination and chemical analysis. The leaf area and dry mass were used to calculate the specific leaf area (SLA=1/LMA) of individual leaves. Leaf chlorophyll

content was determined using the DMSO (Dimethyl sulfoxide) Leaf nitrogen concentration was determined using a N/C analyzer (NC-900, Shimadzu, Kyoto, Japan). Rubisco was measured with SDS-PAGE (Laemmli 1970). The other protein was estimated by the model by Farquhar et al. (1980) and Niinemets and Tenhunen (1997). Leaf anatomical traits were observed by a microscope after the leaves were embedded with epoxide resin. Collected leaves were preserved in 4% glutaraldehyde solution and kept in cool condition with a refrigerator until embedding.

## Results and discussion

### 1) Leaf and crown phenology

Specific pattern of shoot development of tree species also affected the progress in senescence of a crown of trees, i.e. early successional species (birch, alder, ash) in autumn change leaf color from inner part of a crown whereas late successional species progress leaf color from the outer part of a crown (e.g. maple, basswood) (Koike 2004). Alder maintained green leaves at the crown top until early November when early frost comes (Fig. 1). Similar trends in leaf phenology have been found in a forest of northeast U.S. (Bicknell 1982, Bazzaz 1996). Except alder, remobilization of leaf nitrogen in autumn is larger in early successional species than in late successional species. Alder sheds leaves with high nitrogen content (more than 70%) because of symbiotic micro-organism in root (*Frankia* sp.)

Sapling leaves flushed at almost the same time as canopy trees. However, leaf shedding in saplings began one to two weeks later than in canopy trees. Magnolia, a mid successional (or typical gap phase) species, flushed four or five leaves per shoot around late May to early June, and shed its leaves at mid September. Cherry is a late successional species and flushed four or five leaves per shoot at the end of April, keeping the leaves until mid to late October. Carpinus flushed its leaves in mid to late May; it had five leaves per shoot, and these leaves remained green until they were killed by early frost. Oak flushed a few leaves in mid May, and kept its leaves until mid to late September, gradually shedding them in late October. Some oak trees grown in fertile showed the second flush at around July.

The Chl content of ash, birch and elm was highest in mid-canopy (at 15 m) or at the position of the shady crown of the top canopy. Except for October, Chl increased generally to the highest in the sub-layer just below the canopy. Alder maintained a markedly high Chl value even in the latter part of the growing season. In other species, though there was an overall decrease in Chl within the canopy profile, the pattern of Chl distribution did not change throughout the season. The Chl/N ratio in August showed an increase with decreasing rPPF in all species (Koike et al. 2001).

### 2) Seasonal changes in light environment of forest floor

Within a larch forest, rPPF near gaps and on the forest floor showed drastic changes in season with leaf

phenology of upper canopy. Before canopy closure, rPPF averaged 40-60% near the gap and also on the forest floor. Near gaps, rPPF decreased rapidly from May to June, then remained at ca.18% until late September. Larch needles shed in the latter part of October, causing rPPF to increase rapidly. On the forest floor, rPPF diminished with canopy closure, and maintained a low value of ca. 10% until October. In the year of 2000, the reduction in rPPF to ca. 10% was more rapid than in 1999 and 2001, in which years it was ca. 20% at that time. The rate of foliar development was strongly affected by the ambient temperature; e.g. the hot spring in 2000 probably accelerated the rapid development of the canopy cover.

### 3) Seasonal change in photosynthesis in leaves

Seasonal trends in light saturated photosynthetic rate (P<sub>sat</sub>) of regenerated seedlings in a forest floor showed species-specific patterns during the three years (Kitaoka and Koike 2005). Magnolia showed clear seasonal changes in P<sub>sat</sub>, which increased gradually from June to August and then gradually fell toward late September. Cherry and carpinus showed no marked seasonal changes in P<sub>sat</sub>, which remained constant throughout the growing season. Oak displayed a pattern of seasonal change intermediate between that of magnolia, and cherry or carpinus. For oak, P<sub>sat</sub> increased gradually from June to August, then remained constant until the end of September and decreased in October.

In general, P<sub>sat</sub> of deciduous broadleaved trees is closely correlated with LMA (e.g. Koike 1988). Niinemets (2001) has also reported that leaf thickness and LMA correlate positively with the mean monthly air temperature and solar radiation during leaf expansion, but that leaf densities (dry mass per leaf volume) are negatively correlated with precipitation, so that the dry year of 1999 probably had small sized leaves with high density.

Differences in the amount of precipitation during leaf expansion might therefore affect the leaf density in our study, explaining the larger LMA of cherry and carpinus in 1999. The relationship of photosynthesis-nitrogen might also be strongly influenced by these climatic factors, especially temperature (Larcher 2003, Koike et al. 2004, Kitaoka and Koike 2005).

Consequently, the large differences in air temperature and precipitation in the leaf development period between 1999 and the other years may underlie the differences observed in leaf photosynthetic rate, via changes in LMA and leaf physiology (Björkman 1981; Terashima et al. 2001, Kitaoka and Koike 2005).

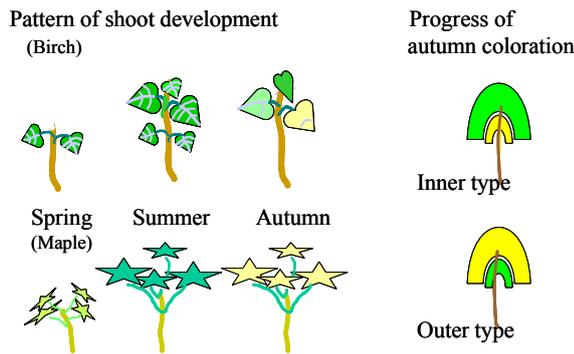


Fig. 1. Progress of autumn coloration of tree crown and pattern of shoot development.

Birch produces succeeding leaves and shed leaves. In contrast, maple flushes all leaves in spring and keeps them until leaf fall. This pattern of shoot development coincides with the progress of autumn coloration of a crown (adopted from Koike 2004).

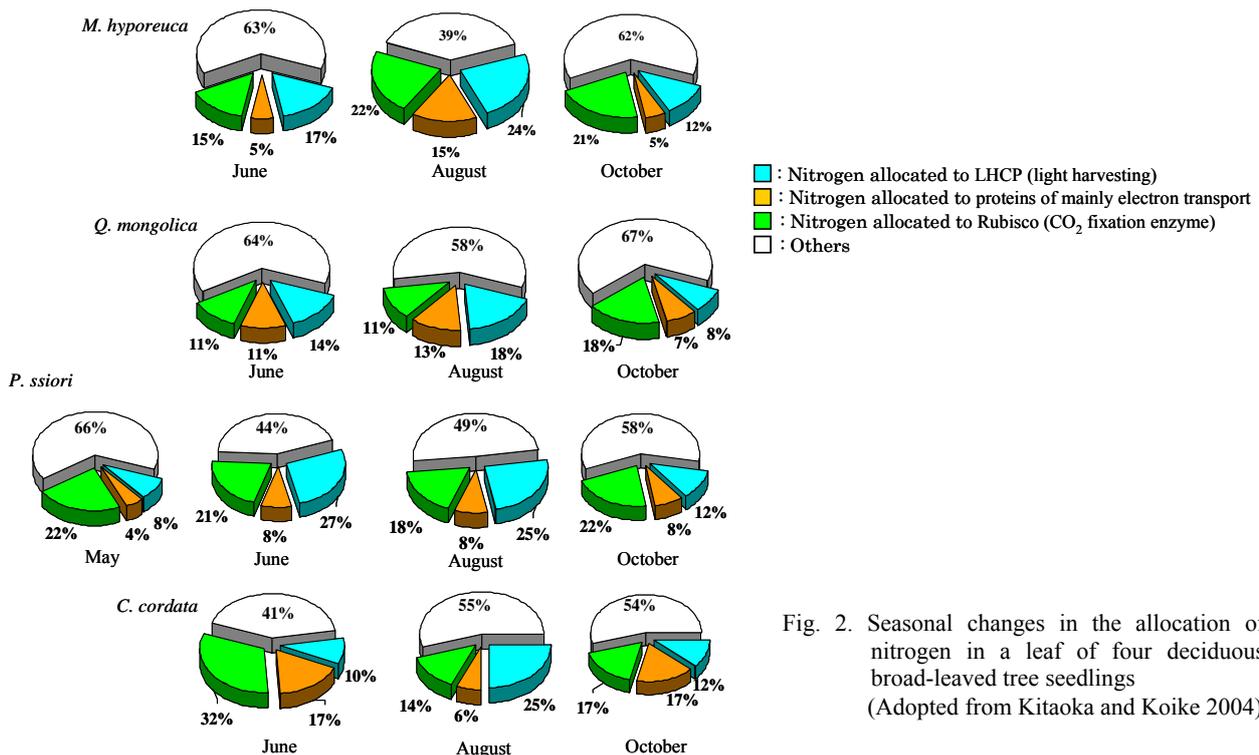


Fig. 2. Seasonal changes in the allocation of nitrogen in a leaf of four deciduous broad-leaved tree seedlings (Adopted from Kitaoka and Koike 2004)

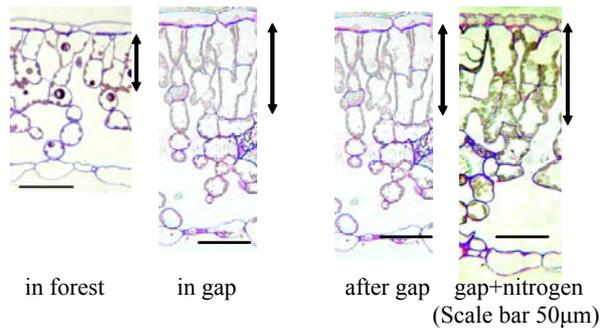


Fig. 3. Example of anatomy of leaves of Magnolia regenerated in a deciduous forest. Leaves collected before and after gap formation and with nitrogen. (Watanabe, Y., unpublished data)

Moreover, biochemical and physiological factors (carboxylation efficiency, RuBP regeneration rate, etc.) and nitrogen allocation would also be influenced by climatic conditions, especially temperature (Makino et al. 1994).

#### 4) Seasonal change in nitrogen allocation of leaves

Nitrogen allocation patterns also show certain relationships to shade tolerance of plants (Bazzaz 1996; Niinemets and Tenhunen 1997, Kitaoka and Koike 2004, Koike et al. 2004). In the magnolia and cherry, the Chl/N ratio increased slightly with canopy closure. This observation suggests that both species can acclimate to shady environment, and might utilize well from sunny to shady light conditions.

With canopy closing at upper layer of forest, nitrogen allocation of leaves of mid successional species increased to light harvesting organs from Rubisco, CO<sub>2</sub> fixing enzyme (Fig. 2). This tendency was clearly found in magnolia saplings in the forest floor (Kitaoka and Koike 2004). In magnolia, increase of the Chl/N ratio in autumn was partly due to its leaf senescence that may be attributed that Chl protein complexes are more difficult to decompose than the other photosynthetic proteins (Larcher 2003). As the progress of leaf senescence, most photosynthetic nitrogen compounds decompose and remobilize to the plant body. The leaf Chl/N ratio should therefore increase, as was found in the late growing season (Koike et al. 2004).

High Psat was found in seedlings of magnolia and oak after the canopy opening with simulated nitrogen deposition through elongation of palisade parenchyma (Fig. 3). With increasing air space in a leaf, CO<sub>2</sub> diffusion in a leaf increase and consequently high Psat may be performed. This tend was also observed in several deciduous broadleaved trees species in summer green forests (Koike et al. 2004).

#### 5) Nitrogen allocation in leaves at high CO<sub>2</sub>

At sunny and ambient [CO<sub>2</sub>] conditions, birch allocated nearly 60% of nitrogen to photosynthetic organs (among them, especially to 43% to Rubisco) whereas under elevated [CO<sub>2</sub>], it allocated 36% of leaf nitrogen to photosynthetic organs (Fig. 4 upper). However, the proportion of Rubisco in birch leaves at

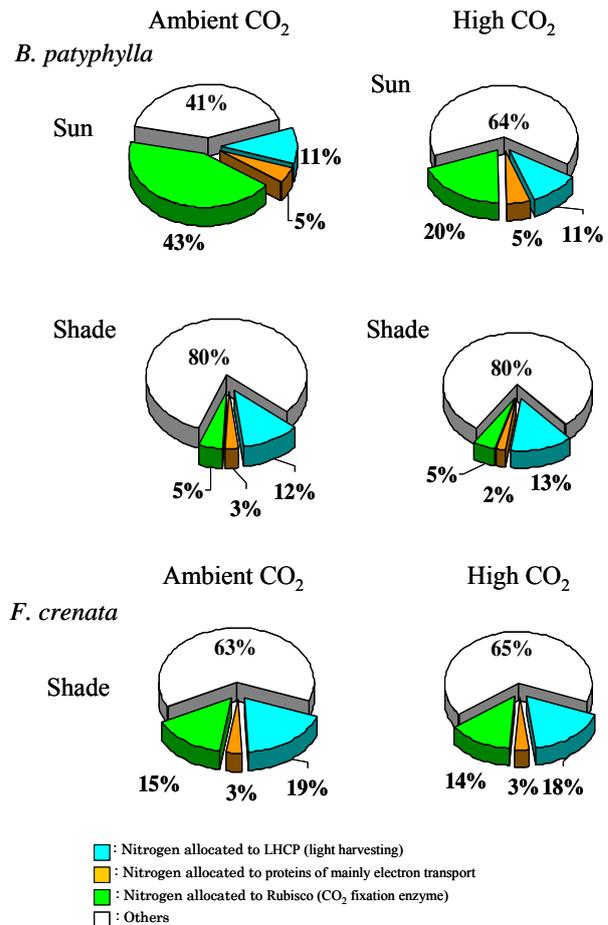


Fig. 4. Changes in nitrogen allocation of a leaf of birch and beech seedlings at different CO<sub>2</sub> and light condition. (after Karatsu et al. 2006)

high [CO<sub>2</sub>] was only 20% of total leaf nitrogen. Although at high [CO<sub>2</sub>] and sunny conditions, birch reduced the allocation of nitrogen to Rubisco but kept constant to the other nitrogen compounds. This tend was observed in light demanding crop plants (Makino 1994).

At shade, percentage of photosynthetic organs was 20~35% in both birch and beech leaves independent of [CO<sub>2</sub>] conditions. At shade, the proportion of leaf nitrogen allocated to more photosynthetic organs in beech than that in birch (Fig. 4 lower), which might be reflected from the original growth traits in both species (Karatsu et al. 2006).

As a result, the pattern of nitrogen allocation in leaves may be regulated by both light and [CO<sub>2</sub>] conditions but light condition may play more critical and essential role of nitrogen allocation pattern.

#### Conclusion

Nitrogen allocation of deciduous broadleaved trees is closely related to the seral traits of each species and their growing conditions. Except alder, remobilization of leaf nitrogen in autumn was larger in early successional species than in late successional species. Alder maintained a symbiotic association with *Frankia*

sp. and shed their leaves containing high nitrogen level. Such accumulations of nitrogen in leaves may be the result of active symbiotic nitrogen fixation. In deciduous forest floors, regenerated seedlings allocated more leaf nitrogen to chlorophyll under shade while they allocated leaf nitrogen more to Rubisco at the canopy opening even in autumn for efficiently use limited nitrogen resources. Nitrogen allocation to Rubisco was reduced by high [CO<sub>2</sub>] but this tendency is more related the original successional traits of tree species.

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