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Comparison of carbon economy during leaf development between short-lived and long-lived leaves

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Introduction

Leaf longevity generally increases in the order of annual herbs, deciduous trees and evergreen trees. Leaf photosynthetic rate on leaf dry mass basis decreases in the same order. The leaf construction cost was thought to be higher in long-lived leaves. However, leaf construction cost on leaf dry mass basis is quite similar, and is about 1.5 g glucose/gd.w. across growth forms [3]. This is probably because long-lived leaves use much energy to produce lignin at the expense of protein synthesis. High leaf cellulose and lignin contents in long-lived leaves would be effective for increasing leaf mechanical strength in long-lived leaves.

On the other hand, during leaf development of evergreen trees, net photosynthetic rate on leaf area basis is often close to zero at full leaf area expansion (FLE), and continues to increase for 10-30 days thereafter [1]. This is contrasted with the well-established pattern for the annual herbs: net photosynthetic rate on leaf area basis maximizes at FLE.

Although leaf construction cost is similar among growth forms, there is a striking difference in construction processes of leaf photosynthesis. These features suggest that leaf construction cost needs to be examined in the light of leaf ontogeny. From carbon and nitrogen contents of the leaf, we can estimate leaf construction cost. Therefore, we examined carbon and nitrogen budget during leaf development. For understanding an overall trend among growth forms, we first selected two contrasting growth forms, annual herbs and evergreen broad-leaved trees.

We used *Phaseolus vulgaris* and an evergreen broad-leaved tree, *Quercus glauca*. For estimating leaf age that leaf changes from heterotrophic (sink) to autotrophic (source) phases, we followed changes in light-photosynthesis relationships, and measured total leaf carbon content (C_{leaf}) in the course of leaf ontogeny. We also measured leaf nitrogen, hemicellulose, cellulose and lignin contents. To study development of leaf mesophyll cells and chloroplasts anatomically, we measured mesophyll surface (S_{mes}) and chloroplast surface (S_c) areas facing the intercellular air spaces on leaf area basis.

Materials and methods

Plant growth conditions

We used an annual herb, *P. vulgaris* L. cv. Yamashiro-kurosando (Fabaceae) plants and *Q. glauca* Thunb. ex Murray (Fagaceae). Plants were grown in a naturally lit plastic greenhouse. *P. vulgaris* plants were grown from seeds in vermiculite in 1.3 L plastic pots. The seeds were selected in a rage of 0.35-0.45 g in weight. *Quercus*

glauca were 4 to 5 year-old saplings that had been grown in 5 L pots under sunny sites for two years. We grew 45 and 10 plants for *P. vulgaris* and *Q. glauca*, respectively. For the experiments, we used primary leaves of *P. vulgaris* that were grown in August to September, or the new leaves flushed in the spring for *Q. glauca*. For studying anatomical changes during leaf development, we used primary leaves of *P. vulgaris* and second-flush leaves of 2-3 year-old saplings of *Q. glauca*.

Measurement of photosynthesis and respiration

We used a portable CO_2 gas exchange system (LI-6400, LI-Cor, Lincoln, NE, USA) for the measurements of rates of leaf photosynthesis and respiration. We measured the dark respiration rate at leaf temperature of 25 ºC, and then generated light-photosynthesis curve. Throughout the measurement of photosynthesis, we kept CO_2 concentration entering the chamber, leaf temperature and leaf to air vapour pressure deficit, 360 μmol mol^{-1}, 25 ºC and less than 1.5 kPa in the chamber, respectively. To estimate daily leaf photosynthetic carbon gain, we calculated daily PPFD changes with hemispherical photographs that were taken with a camera with a fisheye lens.

Measurement of leaf anatomical properties

We cut leaf pieces (about 2 mm^2) from leaves during leaf ontogeny. These were fixed with 2.5% glutaraldehyde and post-fixed with 2% osmium tetroxide. These samples were dehydrated in acetone and propylene oxide series, and embedded in resin. For light microscopy, sections were cut at 0.8 μm thick with an ultramicrotome. Photographs were taken and analyzed with a software (NIH Image). We calculated S_{mes} and S_c as in [1].

Measurement of nitrogen and cell wall constituents

For measurements of nitrogen and cell wall constituent (cellulose, hemicellulose and lignin) contents, we collected leaves and stored at –80 ºC after the measurement of leaf area. They were homogenized in a Na-phosphate buffer. Nitrogen content was determined with Nessler's reagent after Kjeldahl digestion. Protein content was estimated from nitrogen content [6]. Contents of cellulose and hemicellulose were determined according to Sakurai [4] with slight modifications. Lignin content was determined specrtophotometrically according to Morrison [2].

Estimation of carbon cost and construction cost

We collected some leaves and dried at 70 ºC after measurement of leaf area. The dried leaves were milled with a blender. The total leaf carbon content was measured with a CHN analyzer (Perkin Elmer CHNOS analyzer, Perkin Elmer JAPAN). To estimate daily carbon cost of synthesis of protein and of cell wall constituents, carbon content for each constituent was estimated (54%, for protein; 40%, for hemicellulose+cellulose; 40%, for lignin), and the time function of the carbon content per whole leaf was expressed as a logistic equation. Change in C_{leaf} with time was also fitted by this equation. The maximum leaf area was assumed to be 1 m^2 in both species. After the measurement of total carbon content, we estimated leaf mineral concentration from ash, and calculated construction cost [3].

Results and discussions

Net photosynthesis on leaf area basis peaks at about 5 days before FLE in *P. vulgaris* (Fig.1). On the other hand, in *Q. glauca*, net photosynthesis is still close to zero at

FLE and continues to increase for about 10 days. In *Q. glauca*, leaf dry mass per area changed in a similar manner that net photosynthesis changed. $S_cS_{mes}^{-1}$ indicates time difference in development of mesophyll cells and chloroplasts. In expanding leaves of *P. vulgaris*, $S_cS_{mes}^{-1}$ was nearly one: Chloroplasts occupied entire surfaces of leaf mesophyll cells at this stage (Fig.2). In *Q. glauca*, S_{mes} attained the maximum value at FLE, which would mean that the mesophyll cell division and expansion are almost completed at FLE. In *Q. glauca*, $S_cS_{mes}^{-1}$ increased after FLE. Thus, chloroplasts developed slower than mesophyll cells in *Q. glauca*.

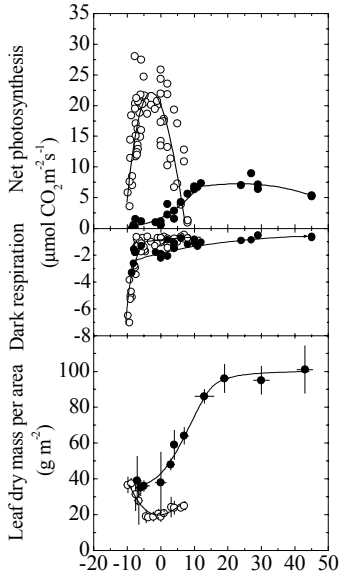

Fig.1

Fig.1. Changes in rates of net photosynthesis and dark respiration on leaf area basis during leaf ontogeny in *P. vulgaris* (○) and *Q. glauca* (●). "Zero" on the abscissa stands for the time of full leaf area expansion.

Fig.2. Changes in mesophyll surface area adjacent to the intercellular air spaces (S_{mes}) and surface area ratio of chloroplasts to mesophyll cells ($S_cS_{mes}^{-1}$) during leaf ontogeny in *P. vulgaris* (○) and *Q. glauca* (●).

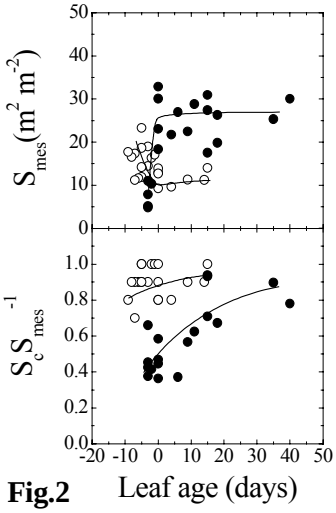

Fig.2

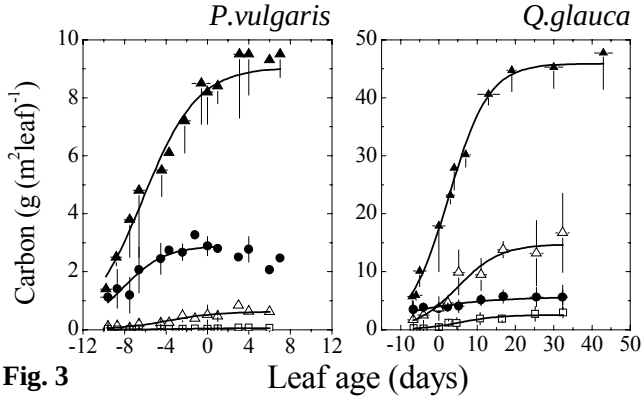

Fig. 3

Fig.3. Changes in total leaf carbon content (C_{leaf}, ″), and carbon contents in protein (●), hemicellulose+cellulose (≥), and in lignin (ϒ) during leaf development in *P. vulgaris* and *Q. glauca*. These values are all expressed per whole leaf. The maximum leaf area was assumed to be 1 m^2 in both species. Note that the scales of two graphs are different. "Zero" on the abscissa stands for the time of full leaf area expansion.

Carbon content for protein accounted for the largest proportion of C_{leaf} during leaf development in *P. vulgaris* (-10 to 0 days) (Fig. 3). On the other hand, in *Q. glauca*, carbon content for hemicellulose+cellulose accounted for the largest proportion of C_{leaf}, particularly in the later stages of leaf development (5 to 40 days). The maximum carbon content in protein in *Q. glauca* was twice as much as that in *P. vulgaris*. On the other hand, expanding leaves of *Q. glauca* showed already high carbon content for protein. The rate of increase in carbon in the protein in *Q. glauca* was slower than in *P. vulgaris* during leaf development (Fig. 4).

In *P. vulgaris*, the transition from sink to source occurred at when the leaf area was about 30% of the maximum. This result holds for the data for most annual herbs [5]. On the other hand, in *Q. glauca*, the transition occurred at about 7 days after FLE, which was 16 days later than that in *P. vulgaris*. These results suggest that the transition from sink to source was considerably retarded in evergreen broad-leaved trees.

This study suggests that leaf mesophyll cell division and expansion in *Q. glauca* were largely dependent on remobilized substances accumulated in stems and roots, and/or photosynthates produced by the old leaves (Figs.2 and 4). 60% of carbon and 76% of nitrogen originated from remobilized substances in *Q. glauca* while only 36% of carbon and 40% of nitrogen in *P. vulgaris* (Fig. 5). Large amount of remobilized substances, particularly nitrogenous compounds, supported the construction of *Q. glauca* leaf. On the other hand, rate of leaf protein synthesis in *Q. glauca* was lower than that in *P. vulgaris* (Fig. 3). In developing leaves, there might be a competition between the cost of synthesis of protein and that of cell wall constituents because construction cost on leaf dry mass basis did not differ throughout their leaf development (data not shown). For plants having long-lived leaves, it would be important to protect their young leaves from mechanical damages (e.g. herbivory) rather than to secure rapid chloroplasts development. These results suggest that there is a striking difference in allocation patterns of carbon and nitrogen for leaf construction between short-lived and long-lived leaves.

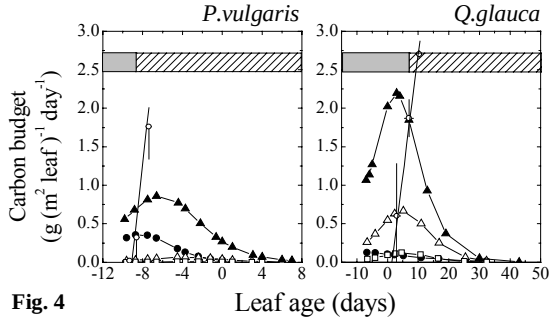

Fig. 4

Fig. 4. Carbon budget during leaf development in *P. vulgaris* and *Q. glauca*. Symbols are the same as in Fig. 3. Solid lines indicate the daily carbon gain by photosynthesis that was estimated from the light photosynthesis curve and dark respiration rate. Shaded and hatched bars indicate sink and source phases, respectively. The maximum leaf area was assumed to be 1 m^2 in both species.

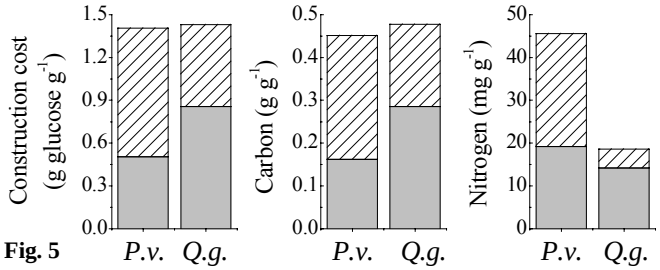

Fig. 5

Fig.5. Construction cost, carbon content and nitrogen content on leaf dry mass basis of photosynthetically mature leaf (at FLE for *P. vulgaris* and 30th day after FLE for *Q. glauca*). Shaded and hatched bars indicate the part of these parameters constructed during sink and source phases, respectively. *P.v.*, *P. vulgris*; *Q.g.*, *Q. glauca.*

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