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Editors

Trees in a Changing Environment

Ecophysiology, Adaptation, and Future Survival

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Trees in a Changing Environment

Ecophysiology, Adaptation, and Future Survival

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Editor's Note

For a variety of reasons, this book project took much longer to completion than planned. It was commenced 4 years ago, and some contributions were received as long as 3 years ago. Fortunately, this volume compiles excellent overviews of current topics in tree environmental physiology and ecosystem process aspects, and the delay does not diminish their value in any way. I am convinced that the contributions in this book will prove of long lasting value in an otherwise fast moving field of research. I would like to take the opportunity to thank all authors, editors, and publishers involved for their excellent contributions and, particularly, for their patience.

Creswick, Australia   Michael Tausz
March 2014

Preface

The Earth's climate is continuously changing and has always changed through time. These changes are based on complex, oscillating cycles that occur on decadal, century, and millennial time scales. Climate shifts are common, marked by ice ages as well as long, warm periods.

There is by now overwhelming evidence that human activities have altered natural climatic cycles (Stocker et al. 2013). Although atmospheric chemistry changes (in CO_2, CO, O_3, CH_4) have occurred in the past due to natural causes, the current and expected future atmospheric composition is unlike any in the past due to anthropogenically generated air pollution (in addition to the above: NO_x and tropospheric O_3, double the concentration of the pre-industrial era).

In computer-based models (general circulation models, GCMs), rising concentrations of greenhouse gases have resulted in an increase in air temperature and instabilities in weather. Warmer air holds more water, and it evaporates from all surfaces: soil, vegetation, and open water. In other areas, there will be flooding, just as deleterious as drought to maladapted species. Because every component of ecosystems responds to temperature and water, current ecosystems are and will continue to change in response to increases in temperature, increases in evaporation, and weather instabilities (extremes in temperature and precipitation, its form, and when the extremes occur). Evidence for climate change has already been reported in thousands of publications, in locations distributed throughout the globe. These changes, as well as predicted future changes, are predicted with high confidence on a global scale, yet may differ considerably from place to place (Stocker et al. 2013).

An increase in air temperature of 1–1.5 °C above the mean for 1850–1900 is highly likely by mid-century. In addition to the direct effect of increasing air temperature on water balance, global circulation models predict different amounts of precipitation (Stocker et al. 2013). The greatest threat to ecosystems is increased frequency, duration, and extremity of water availability (from drought to flooding) and temperature (unusual timing and duration of cold snaps, prolonged heat spells) that will disrupt function, survival, and distribution of plants, animals, insects, and pathogens adapted to a past, or at best the current environment. In addition, air

pollution effects on ecosystems need to be considered over the long term, especially with regard to the fertilizing effects of CO_2 and nitrogen deposition, and the deleterious and CO_2-negating effects of tropospheric O_3 on carbon uptake and its allocation. Although generalized approaches to managing ecosystems for climate change may be developed, the novel combinations of atmospheric chemistry, temperature, water availability, and the instabilities and extremities in weather will require novel, place-based land management approaches for ecosystems.

At a time when much of the world seems to be discussing climate change, one might ask, 'why another book anticipating effects of climate change?' Firstly, because trees are such long-lived organisms they depend on the acclimation potential of the individuals throughout their lifetime for their survival. Adaptive evolutionary change is slow in species with long generation cycles; hence trees are particularly vulnerable to rapid environmental changes. It is therefore even more important to understand the life functions of trees and the function of forests to underpin possible adaptive management strategies, and these will most likely be different from strategies under consideration for annual or short cycle natural or cropping systems. Secondly, most treatises on climate change effects on biological systems are CO_2-centric: they emphasize CO_2 fertilization and CO_2-induced increased temperatures, accompanying decreases in water availability (in general), but increased plant water use efficiency. We have included the interactive effects of elevated CO_2, the physical environmental effects of green-house gas accumulation, and the *source* of the CO_2: atmospheric chemical changes of air pollution (CO_2, O_3, NO_x, and nitrogen deposition). This is a fundamental consideration that many of the discussions on climate change have ignored, or considered only in isolation (with some noted exceptions, see Emberson et al. 2000, who advocated integration of the effects of these components in a process-based model). Due to the difficulty and the magnitude of experimental studies with multiple factors, there are few field studies that have accomplished two abiotic interactive factors (such as CO_2 x temperature, Kellomäki et al. 2000, CO_2 x N amendment, Pääkkönen and Holopainen 1995, O_3 x CO_2 Karnosky et al. 2003, or O_3 x N amendment, Watanabe et al. 2006), let alone many environmental and biological factors over the lifetime of trees and within the complexity of forest ecosystems. Some studies along environmental gradients with carefully matched sites (e.g., high N deposition, drought stress, and moderate O_3 exposure vs. high N deposition and moderate O_3 exposure alone, Miller and McBride 1999) can provide an insight into multiplicative effects. However, we are still restricted to the *current* range in conditions and responses of extant trees that established in a past climate: 80–250+ years ago. The future holds an unprecedented combination and quantity of atmospheric chemicals, and it is as yet unclear whether and which current species or populations of trees are sufficiently equipped to cope with such conditions.

Our ecosystems are already and unequivocally (Stocker et al. 2013) experiencing environmental and climate change, and forests and other tree dominated ecosystems are likely to be severely affected. In this book, the authors have thoughtfully reviewed and described constituent functions and processes that will help us understand tree responses to the complex, concurrent effects of environmental

stresses imposed by climate change, and its ultimate source, air pollution. In many cases they have challenged current theory on expected responses, and in all cases they have contributed their expert knowledge on tree and forest ecosystem response to environmental change: an integrated, qualitative assessment. We offer this comprehensive analysis of tree responses and their capacity to respond to environmental changes to give us better insight as to how to plan for the future.

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References

Emberson LD, Simpson D, Tuovinen J-P, Ashmore MR, Cambridge HM (2000) Modeling and mapping ozone deposition in Europe. Water Air Soil Pollut 130: 577–582

Karnosky DF, Zak DR, Pregitzer KS, Awmack CS, Bockheim JG et al (2003) Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. Funct Ecol 17: 289–394

Kellomäki S, Wang K-Y, Lemettinen M (2000) Controlled environment chambers for investigating tree response to elevated CO_2 and temperature under boreal conditions. Photosynthetica 38: 69–81

Miller PR, McBride JR (1999) Oxidant air pollution impacts in the Montane forests of Southern California: a case study of the San Bernardino Mountains. Ecological Studies 134, Springer, New York

Pääkkönen E, Holopainen T (1995) Influence of nitrogen supply on the response of clones of birch (*Betula pendula* Roth) to ozone. New Phytol 129: 595–603

Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) (2013) Climate Change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, New York

Watanabe M, Yamaguchi M, Iwasaki M, Matsuo N, Naba J, Tabe C, Matsumura H, Kohno Y, Izuta T (2006) Effects of ozone and/or nitrogen load on the growth of *Larix kaempferi*, *Pinus densiflora* and *Crytomeria japonica* seedlings. J Jpn Soc Atmos Environ 41: 320–334

Contents

Chapter 1
Resource Allocation and Trade-Offs in Carbon Gain of Leaves Under Changing Environment

Kouki Hikosaka, Yuko Yasumura, Onno Muller, and Riichi Oguchi

Abstract In leaf canopies, environmental conditions such as light availability and temperature vary spatially and temporally. Plants change leaf traits such as leaf nitrogen content, leaf mass per area, leaf anatomy, photosynthetic capacity, and organization of the photosynthetic apparatus in response to the change in conditions. These changes occur because a trait that is optimal under a certain condition is not advantageous under others. When growth irradiance is high or air temperature is low, plants invest more nitrogen into ribulose-1,5-bisphosphate carboxylase (Rubisco) rather than photosystems. Leaf nitrogen content is high under such conditions because nitrogen content that maximizes nitrogen use efficiency of daily carbon gain is higher under higher irradiance or lower temperature conditions. Leaf anatomy constrains the maximal rate of photosynthesis: leaves with higher photosynthetic rate should be thicker to allot more chloroplasts on mesophyll surface. To increase maximal photosynthetic rate after gap formation, shade leaves of some species are thicker than the minimum required for the photosynthetic rate, allowing further increase in chloroplast volume.

1.1 Introduction

In leaf canopies, there are spatial and temporal variations in photosynthetically active photon flux density (PFD) and temperature. Air temperature and PFD change seasonally especially in higher latitudes. PFD decreases with depth within canopies

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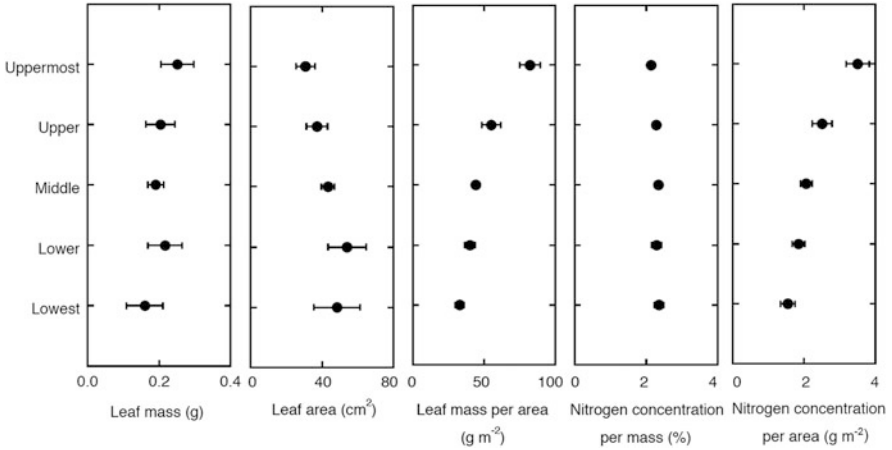
Fig. 1.1 Gradient of leaf traits in a canopy of *Fagus crenata* (Drawn from data shown by Yasumura et al. (2005) and unpublished data (Y. Yasumura))

and often varies by two orders between the top and bottom of dense canopies (Monsi and Saeki 1953). Gap formation, which is an important event in forest ecosystems, greatly increases PFD in understorey. Various leaf traits exhibit significant changes in response to such environmental changes. For example, leaf nitrogen content is highest in the leaves at the top of canopy (Fig. 1.1). As a result of these variations, photosynthetic activity of leaves varies greatly across canopy layers and seasonal environment.

Responses in leaf traits to environmental change is an important information for correct prediction of carbon flow in forest ecosystems (Baldocchi and Harley 1995; Wilson et al. 2001; Ito 2010). Why do leaf traits change in response to environmental changes? This may be because a leaf that is adapted to a certain environment is not necessarily adapted to other environments. If a resource is allocated to improve one function, it inevitably causes a reduction in other functions, i.e., there are trade-offs in resource allocation.

Here is a review of photosynthetic acclimation to spatial and temporal heterogeneity in environment. We particularly focus on light and temperature as important environmental factors. We discuss trade-offs in resource allocation and its relation to optimization of photosynthetic performance.

1.2   Trade-Off in Nitrogen Allocation Among Photosynthetic Components

Nitrogen is one of the most important factors that limit plant growth in many ecosystems (Aerts and Chapin 2000). Even under non-limiting conditions, nitrogen acquisition requires carbon costs, which are utilized for development and

maintenance of root systems and uptake, assimilation, and translocation of nitrogen. Therefore efficient use of nitrogen is an important strategy to survive, grow and reproduce under natural environments (Aerts and Chapin 2000).

The photosynthetic apparatus is the largest sink of nitrogen in plants; approximately half of leaf nitrogen is invested in photosynthetic proteins (Evans and Seemann 1989; Hikosaka 2010). The photosynthetic apparatus consists of various proteins. Photons are absorbed by chlorophylls (chl) associated with photosystems I and II (PSI and PSII) and the excitation energy is utilized for electron transport from water to NADPH and for proton transport across thylakoid membranes to produce ATP. NADPH and ATP are utilized in Calvin cycle to produce sugars. The first step of CO_2 fixation is catalyzed by ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), where CO_2 is bound to ribulose-1,5-bisphosphate (RuBP) forming 3-phosphoglyceric acid (PGA). Triose phosphate (TP) is then produced by using ATP and NADPH. Some of TP is transported to the cytosol and used for sucrose synthesis, the remainder is used for the regeneration of RuBP. From the viewpoint of energy utilization, the photosynthetic apparatus can be divided into light harvesting (photosystems) and light use (other parts). Under low light, light harvesting limits photosynthesis, while light use is the limiting process under high light.

The organization of the photosynthetic apparatus changes depending on growth photon flux density (PFD). For example, the ratios of Rubisco to chl and of chl *a* to chl *b* increase with increasing growth PFD. Such acclimation has been reported not only for herbaceous species (Boardman 1977; Anderson 1986; Terashima and Evans 1988) but also for woody species (Hikosaka et al. 1998; Fig. 1.2). Within-canopy variation in the photosynthetic apparatus has also been shown along light gradients (Niinemets 1997; Niinemets et al. 1998; Warren and Adams 2001; Laisk et al. 2005; Turnbull et al. 2007; Fig. 1.3).

Changes in the organization of the photosynthetic apparatus are related to the role of each component in photosynthesis. Photosynthetic rates exhibit a saturating curve against PFD. When PFD is low, photosynthetic rate linearly increases with increasing light, whereas it saturates at high PFD. The initial slope of the curve is the product of quantum yield and light absorption, the latter of which increases with increasing chl content of the leaf (Gabrielsen 1948). The light-saturated rate of photosynthesis (P_{max}) is, on the other hand, related to the content of other photosynthetic proteins (von Cammerer and Farquhar 1981; Evans 1983; Makino et al. 1983). Particularly, Rubisco content is important because it catalyzes the limiting step of photosynthesis when CO_2 concentration is low under saturating light. Thus nitrogen should be invested more into the light harvesting part under low PFD and to the light use part such as Rubisco under high PFD (Evans 1989).

Hikosaka and Terashima (1995) developed this idea further and constructed a comprehensive model to predict the optimal nitrogen partitioning among photosynthetic components. In this model photosynthetic components were categorized into five groups: Group I, Rubisco; Group II, electron carriers, ATP synthetase, and Calvin cycle enzymes other than Rubisco; Group III, core complex of PSII (PSII core); Group IV, core complex and light harvesting chl-protein complex of PSI, and
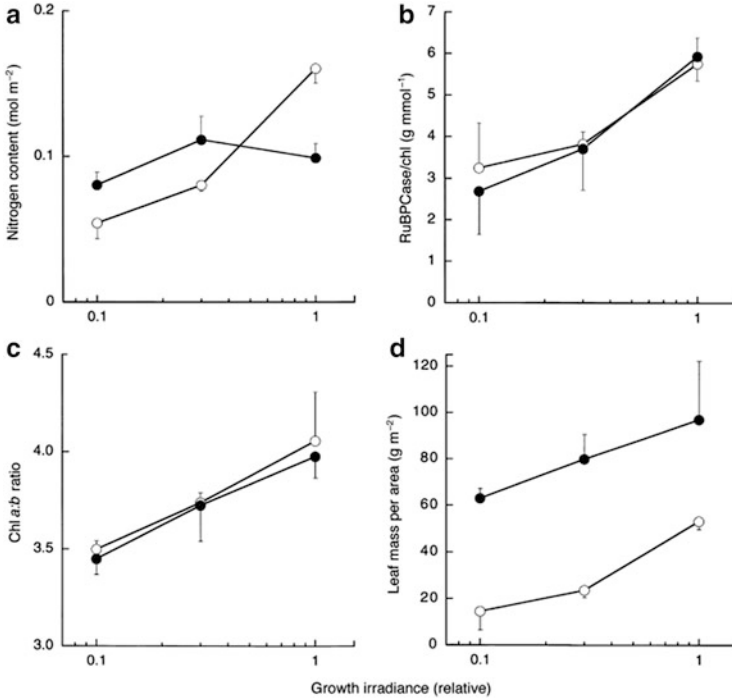
Fig. 1.2 Photosynthetic acclimation in *Chenopodium album* (annual herb; *open symbols*) and *Quercus myrsinaefolia* (evergreen tree*; closed symbols*) grown at different light regimes. RuBCase = Rubisco (Redrawn from Hikosaka et al. 1998)

Group V, light harvesting chl-protein complex of PSII (LHCII). The nitrogen cost for each group was calculated from published data. P_{max} was assumed to be a function of amount of proteins belonging to Group I, II and III. The initial slope was assumed to be a function of chl content. Optimal nitrogen partitioning that maximizes daily carbon gain was calculated. It considerably changed with PFD conditions. Under high PFD, daily carbon gain increases with increasing nitrogen allocation to proteins related to P_{max}, while under low PFD carbon fixation is high when nitrogen is allocated more to photosystems (Fig. 1.4). These results are consistent with the observations that leaves allocate more nitrogen to Rubisco than to chl at higher PFD (Fig. 1.2). Optimal nitrogen investment is higher in PSII core than in LHCII at higher PFD. This is because more PSII core is necessary for higher P_{max}. On the other hand, greater amount of LHCII is only advantageous under low PFD because nitrogen cost of chl (the ratio of chl to N in each group) is higher in LHCII than in PSII. Because most of chl *b* is associated with LHCII, this result explains why the chl *a/b* ratio increases with increasing growth PFD (Fig. 1.2).

Hikosaka and Terashima (1996) applied this model to plants of a sun (*Chenopodium album*) and a shade (*Alocasia macrorrhiza*) species grown under
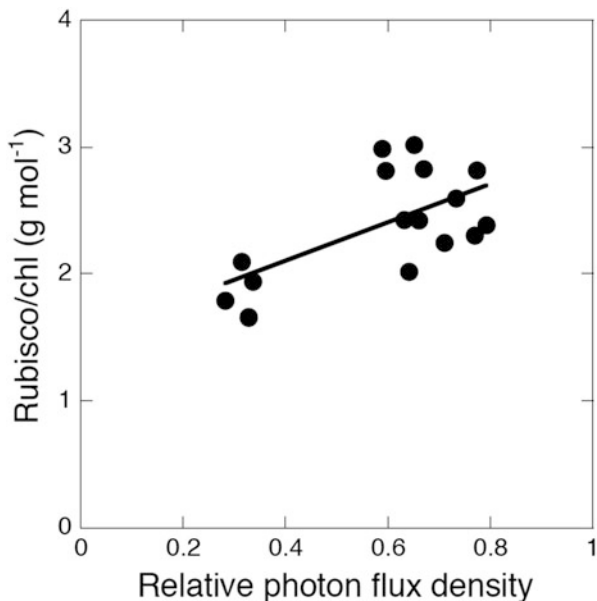
Fig. 1.3 The ratio of Rubisco to chl as a function of the intercepted irradiance in canopy leaves of *Quercus crispula* (Unpublished data (O. Muller))
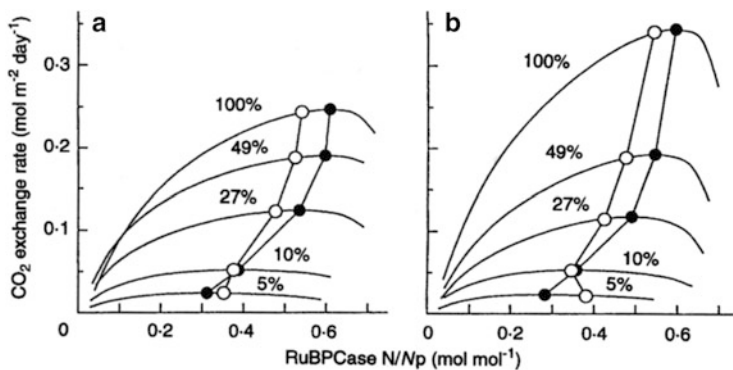
Fig. 1.4 Daily carbon gain as a function of N partitioning in *Alocasia macrorrhiza* (perennial herb; (**a**)) and *Chenopodium album* (annual herb; (**b**)) grown under various PFD (5–100 % of full sunlight). *Open* and *closed symbols* denote actual and optimal nitrogen partitioning (Redrawn from Hikosaka and Terashima 1996)

various PFD conditions. Figure 1.4 shows the effect of nitrogen partitioning on daily carbon gain. There was an optimal nitrogen allocation to Rubisco and it increased with increasing growth PFD (closed circles). Similar to the optimum, actual nitrogen partitioning to Rubisco also increased with increasing growth PFD

(open circles). Difference between optimal and actual nitrogen partitioning was small except for *C. album* plants grown at the lowest PFD (5 % of full sunlight). These results suggest that plants can adjust nitrogen partitioning among photosynthetic components nearly optimally to different light regimes, though sun species might be unable to do so in very low PFD.

Similar changes in nitrogen partitioning occur when growth temperature changes. With decreasing growth temperature, for example, the Rubisco/chl and chl *a*/*b* ratios increase (Hikosaka 2005; Yamori et al. 2009). This may be because of the difference in temperature dependence between the light harvesting and light use parts. As with other enzyme activities, activity of Calvin cycle enzymes is sensitive to temperature and is generally lower at low temperatures. In contrast, photochemical reactions are insensitive to temperature, and consequently the initial slope of light response curve is less affected by temperature. At low temperatures, therefore, proteins related to the light use part should be enhanced to keep the balance between the light harvesting and use.

In temperate climates at mid-latitudes, temperature and light climate vary strongly during the year (Fig. 1.5). In winter, the air temperature is around freezing point and the PFD is lower with shorter day lengths than in summer when air temperature is around 25 °C (Röhrig 1991). In the understorey of deciduous forests, large changes in PFD occur due to sprouting and fall of canopy leaves in spring and autumn, respectively. Leaves of evergreen species in the understorey of such forests are exposed to large changes in light and temperature conditions over the year, which may affect leaf functioning.

Muller et al. (2005) investigated seasonal change in the photosynthetic traits of leaves of an evergreen understory shrub *Aucuba japonica* grown at three different light regimes: gap, understory of deciduous forest, and understory of evergreen forest. They applied multiple regression to evaluate quantitative contribution of temperature and PFD to the photosynthetic acclimation (Fig. 1.5). The Rubisco/chl ratio was significantly correlated both with air temperature and PFD as well as the chl *a*/*b* ratio. Across sites PFD had stronger effects than air temperature, while within a site temperature had stronger effects on photosynthetic acclimation. It was concluded that the photosynthetic apparatus is strongly affected by the prevailing PFD at the time of leaf development. Within a given light regime, however, the balance between Rubisco and chl responds mainly to temperature and to a lesser extent to PFD.

Apart from the trade-off mentioned above, there is another trade-off between nitrogen allocation between two processes, carboxylation and regeneration of RuBP. At low CO_2 concentrations under saturated light, RuBP carboxylation is the limiting step of photosynthesis, while RuBP regeneration limits photosynthesis at high CO_2 concentrations. Thus to increase photosynthetic rates at low CO_2 concentrations nitrogen should be more allocated to Rubisco, whereas it should be more to RuBP regeneration processes (Group II and III) at high CO_2 concentrations (Hogan et al. 1991; Sage 1994; Webber et al. 1994; Medlyn 1996; Hikosaka and Hirose 1998; Fig. 1.6). Hikosaka and Hirose (1998) theoretically showed that elevated CO_2 (from 350 to 700 μmol mol^{-1}) increased daily carbon gain by 40 %

Fig. 1.5 Seasonal changes in PFD (**a**), temperature (**b**), leaf nitrogen content per area (**c**), Rubisco content (**d**), chl *a/b* ratio, and Rubisco/chl ratio in *Aucuba japonica*, an evergreen shrub, growing three light regimes (*squares*, gap; *circles*, deciduous understory; *triangle*, evergreen understory) (Redrawn from Muller et al. 2005)

Fig. 1.6 Effects of nitrogen allocation in the photosynthetic apparatus on CO_2 dependence of photosynthesis. See text for detail
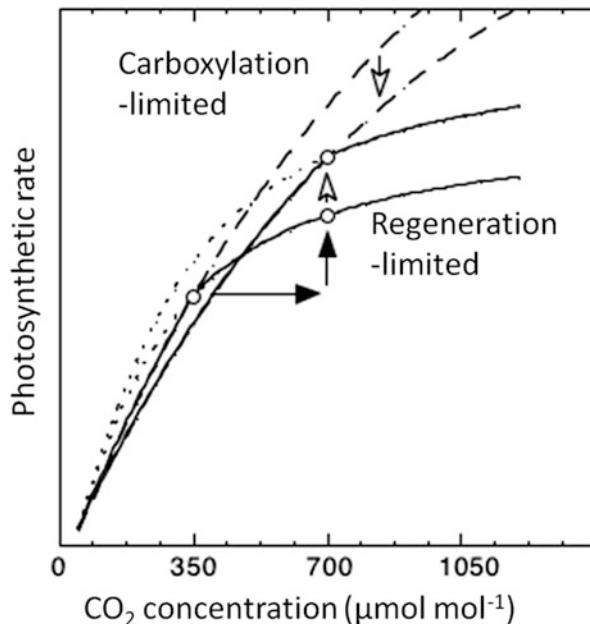
when nitrogen partitioning is optimal for 350 μmol mol^{-1} CO_2, while it increased by 60 % when nitrogen is reallocated to maximize photosynthesis at 700 μmol mol^{-1}. This prediction is consistent with the result on transgenic rice with decreased Rubisco content (Makino et al. 1997). When leaves with similar nitrogen content were compared, the transgenic leaves had lower photosynthetic rates than those of wild type at low CO_2 concentrations, but the opposite was the case at high CO_2 concentrations.

In experimental studies, nitrogen allocation to Rubisco and RuBP regeneration processes has been evaluated as V_{cmax} (maximum carboxylation rate) and J_{max} (maximum electron transport rate, Farquhar et al. 1980), respectively. In the 1990s, it was believed that the balance between J_{max} and V_{cmax} was not significantly affected by growth CO_2 concentrations (e.g., Sage 1994; Medlyn et al. 1999). However, recent meta-analyses of FACE (free air CO_2 enrichment) studies have indicated that the J_{max}/V_{cmax} ratio changed significantly with growth CO_2 concentration (Long et al. 2004). Osada et al. (2010) studied photosynthetic traits of *Polygonum sachalinense* plants (a perennial herb) growing around natural CO_2 springs where plants had been exposed to high CO_2 concentrations for the long term and found a significant effect of CO_2 concentration on the J_{max}/V_{cmax} ratio. These results suggest that these plants can alter the balance between carboxylation and regeneration of RuBP depending on growth CO_2 concentration. However, the alteration in actual plants does not seem to be optimal in a quantitative sense. For example, in the study of Osada et al. (2010), the J_{max}/V_{cmax} ratio increased by only 5–6 % when the current CO_2 concentration doubled, and increased by 5 % in FACE experiments at ambient CO_2 + 200 μmol mol^{-1} CO_2 (Ainsworth and Long 2005).

These values are much smaller than the theoretical prediction of a 40 % increase in the J_{max}/V_{cmax} ratio with a doubling of the current CO_2 concentration (Medlyn 1996).

The balance between carboxylation and regeneration of RuBP changes also with temperature. Potential rate of RuBP regeneration exponentially increases with increasing temperature, whereas that of RuBP carboxylation depends less on temperature because of Rubisco kinetics (Fig. 1.7). When temperature is low, therefore, increased nitrogen allocation to RuBP regeneration processes is beneficial (Hikosaka 1997; Hikosaka et al. 2006; Fig. 1.7). Hikosaka et al. (1999a) found that *Quercus myrsinaefolia*, an evergreen tree, realized such changes in the balance between carboxylation and regeneration of RuBP. Hikosaka (2005) found that *Plantago asiatica*, a perennial herb, invested more nitrogen in RuBP regeneration at low growth temperature (Fig. 1.8). However, it has been indicated that some species alter the balance but the others do not (Hikosaka et al. 2006). For example, Hikosaka et al. (2007) studied temperature dependence of photosynthesis in canopy leaves of *Quercus crispula*, a deciduous tree, which did not show seasonal change in the J_{max}/V_{cmax} ratio. Onoda et al. (2005) showed that the J_{max}/V_{cmax} ratio exhibited a seasonal change in seedlings of *Polygonum cuspidatum*, a perennial herb, but not in those of *Fagus crenata*, a deciduous tree. Recently, Yamori et al. (2010) compared temperature acclimation in cold-sensitive and tolerant crop species, the latter of which tended to show greater changes in the J_{max}/V_{cmax} ratio depending on growth temperatures.

1.3 Nitrogen Use Efficiency of Daily Carbon Gain at Leaf and Canopy Levels

In many canopies, there is a vertical gradient of leaf nitrogen content per unit area (N_{area}) (De Jong and Doyle 1985; Hirose and Werger 1987b; Hollinger 1989; Evans 1993; Ellsworth and Reich 1993; Anten et al. 1998; Niinemets 1997; Niinemets et al. 2001; Kikuzawa 2003; Wright et al. 2006; Migita et al. 2007; Yasumura et al. 2005; Fig. 1.1). This gradient is formed mainly in response to the gradient of light availability. This has been proved mainly using herbaceous canopies. For example, the gradient of N_{area} is steeper in a denser than in a scarce canopy (Hirose et al. 1988). N_{area} in vine species where PFD was manipulated, changes depended on PFD (Hikosaka et al. 1994). The gradient of N_{area} is steeper in canopies that have steeper light gradients (Anten et al. 1995, 2000; Ackerly and Bazzaz 1995).

Because almost half of leaf nitrogen is invested in the photosynthetic apparatus, photosynthetic rate is related to N_{area} (Evans 1989; Evans and Seemann 1989; Hikosaka 2010). In particular, there is a strong correlation between P_{max} and N_{area} (Hirose and Werger 1987a; Evans 1989; Hikosaka et al. 1998; Hikosaka 2004; Niinemets et al. 2001; Warren and Adams 2001; Fig. 1.9a). Dark respiration rate is also positively correlated with N_{area} (Hirose and Werger 1987a; Anten
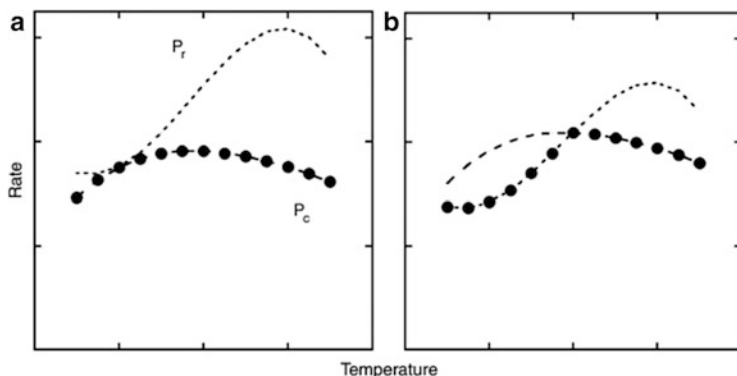
Fig. 1.7 Effect of nitrogen allocation on temperature dependence of photosynthesis. *Broken* and *dotted lines* represent potential rate of Rubisco- (P_c) and RuBP regeneration-limited (P_r) photosynthesis, respectively. *Closed circles* are realized photosynthetic rate, defined as the lower rate of Rubisco- and RuBP regeneration-limited photosynthesis (Redrawn from Hikosaka et al. 2006)

et al. 1995; Hikosaka et al. 1999b). On the other hand, correlation between photosynthetic rate at low light and N_{area} is generally weak (Hirose and Werger 1987a). Weak but significant correlation has been observed between the initial slope and N_{area} in some studies (Hirose and Werger 1987a; Hikosaka et al. 1999b), but not in others (Anten et al. 1995).

As a result of these correlations, daily carbon gain depends on N_{area}; daily carbon gain increases with increasing N_{area} until its optimum and gradually declines due to increasing respiration rate (Hirose and Werger 1987a; Fig. 1.9b). Each curve has two optima. One is the N_{area} that maximizes daily carbon gain (N_{optCER}; A in Fig. 1.9b) and the other is the N_{area} that maximizes nitrogen use efficiency of carbon gain (daily carbon gain per unit leaf nitrogen, daily NUE) (N_{optNUE}; B in Fig. 1.9b, c) (Hirose 1984; Hirose and Werger 1987a; Hikosaka and Terashima 1995). N_{area} values at both optima increase with increasing PFD (Fig. 1.9b, c), which explains why N_{area} is higher in upper leaves.

The optimal N_{area} that maximizes daily NUE (N_{optNUE}) implicitly assumes a trade-off between leaf area and N_{area}. When the amount of nitrogen for a leaf is limited, plants have two choices: one is increasing leaf area, which inevitably reduces N_{area}, and the other is increasing N_{area} at the expense of leaf area. N_{optNUE} is truly optimal when photosynthesis is limited only by nitrogen. However, it is not the case if there are other limitations such as carbon supply. Hikosaka and Terashima (1995) discussed that N_{area} will be closer to the N_{optCER} when nitrogen is more available, while it will be closer to N_{optNUE} when nitrogen is more limited. This is consistent with the experimental results of leaves of spinach (*Spinacia oleracea*) (Hikosaka and Terashima 1995; Terashima and Hikosaka 1995).

N_{area} exhibits seasonal change. In deciduous trees, N_{area} increases after unfolding and reaches maximum in mid summer (Wilson et al. 2000, 2001; Hikosaka et al. 2007; Fig. 1.10). Some of leaf nitrogen is resorbed and others

Fig. 1.8 Nitrogen partitioning in *Plantago asiatica* (perennial herb) leaves grown at high-light with low-temperature (*closed circle*), high-light with high-temperature (*open circle*), and low-light with low-temperature (*closed square*). FBPase (stroma fructose-1,6-bisphosphatase) activity represents nitrogen investment in the RuBP regeneration process. RuBPCase = Rubisco
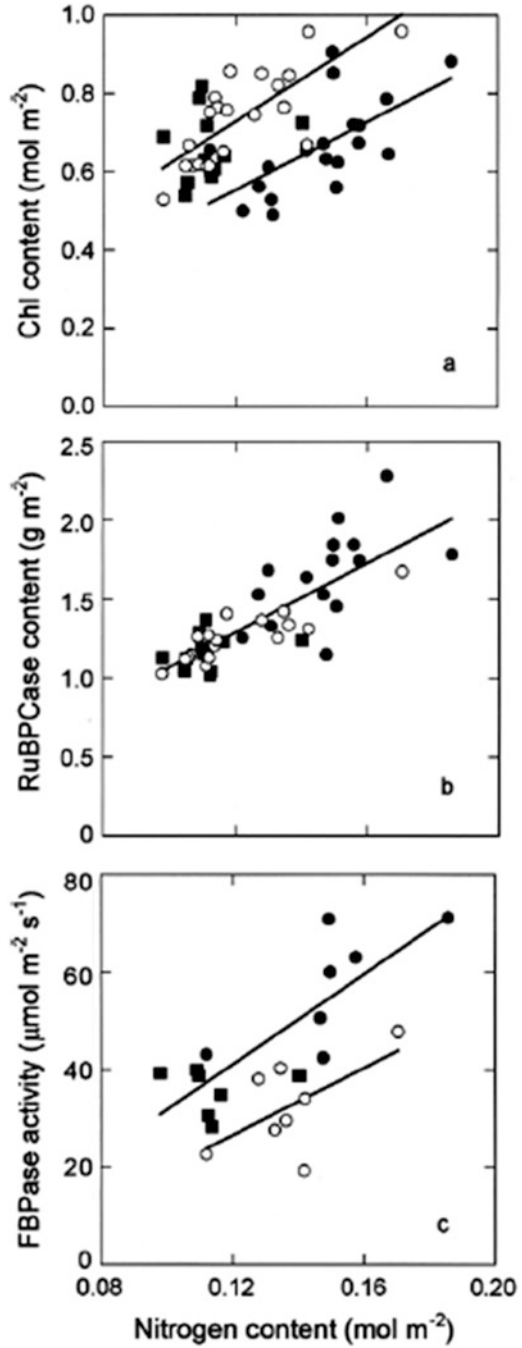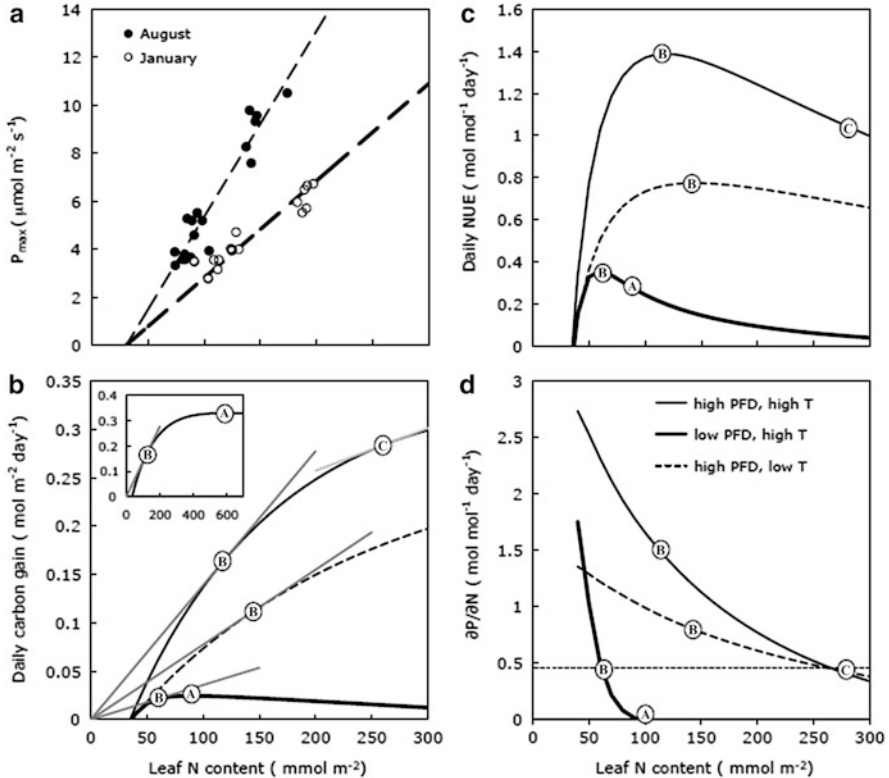
Fig. 1.9 Dependence on leaf nitrogen content (N_{area}) of the light saturated rate of photosynthesis (P_{max}; **a**), daily carbon exchange rate (CER; **b**), daily nitrogen use efficiency (NUE, CER per leaf nitrogen; and slope of the tangent of daily CER ($\partial P/\partial N$; **d**) in *Aucuba japonica*. *Closed* and *open symbols* in a are data obtained in summer (August) and winter (January) at the growth temperature. Daily CER was calculated based on the data shown in (**a**). *Continuous, dotted,* and *thick lines* denote values at summer under high light conditions, those at winter under high light conditions, and those at summer under low light conditions, respectively. The *circle A* and *B* denote the N_{area} that maximizes daily CER and daily NUE, respectively. In the *circle "C"*, $\partial P/\partial N$ of high light leaves is identical to that of low light leaves in *"B"*, indicating optimal allocation of nitrogen between these two leaves. Calculated with data in Muller et al. (2011)

drop with dead leaves (Yasumura et al. 2005). Yasumura et al. (2005) showed that nitrogen resorption efficiency in leaves was not different among layers, though N_{area} was very different.

In evergreen trees, N_{area} is generally highest in winter (Fig. 1.5). Muller et al. (2005) applied multiple regression analysis to analyze effect of PFD and temperature on N_{area} and showed that both PFD and temperature significantly affected N_{area}; leaf N_{area} was high when PFD was high and temperature was low. Experimental studies have also shown that N_{area} is higher at lower temperature regimes (Hikosaka 2005; Yamori et al. 2009).

Fig. 1.10 Seasonal changes in (**a**) mean leaf mass per unit area (LMA), (**b**) leaf nitrogen concentration per unit mass (N_{mass}) and (**c**) leaf nitrogen concentration per unit area (N_{area}) in canopy leaves of *Quercus crispula* in 2001 (*closed circle*) and 2002 (*open circle*). Bars are standard deviations. Polynomial curves are fitted for (**a**) ($r^2 = 0.95$, $P < 0.05$) and (**c**) ($r^2 = 0.99$, $P < 0.05$) (Redrawn from Hikosaka et al. 2007)
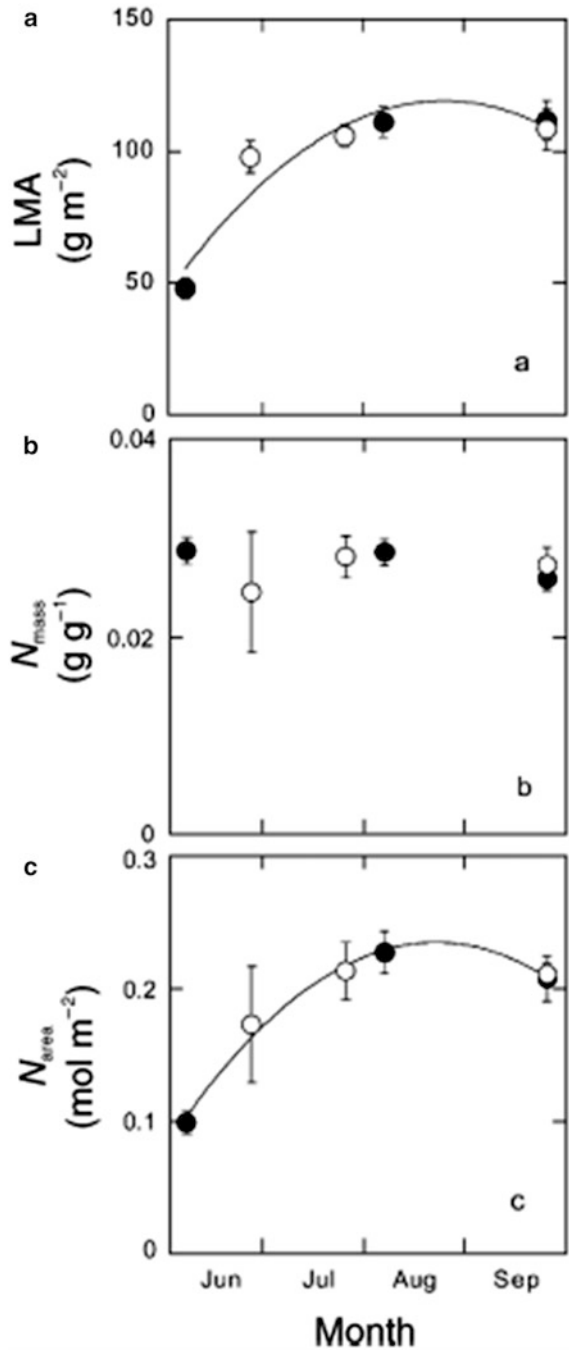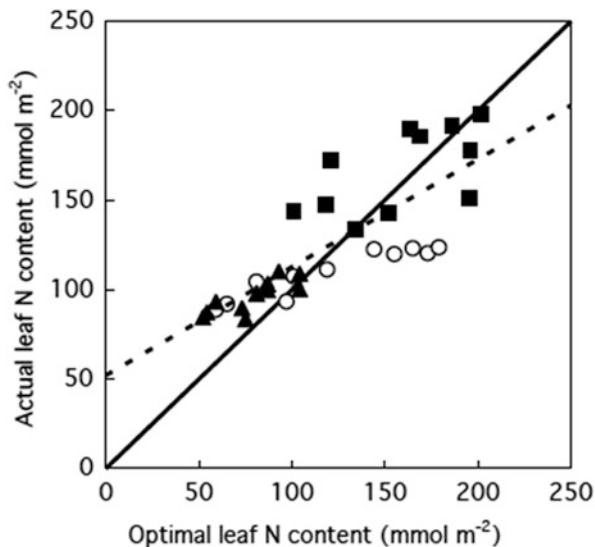
Fig. 1.11 Optimal leaf nitrogen content in relation to the actual leaf nitrogen content in *Aucuba japonica* growing in a gap (*closed squares*), under a deciduous canopy (*open circles*) and evergreen canopy (*closed triangles*) with linear regression line (*broken line*; $r^2 = 0.65$) and 1:1 line (*solid line*) (Redrawn from Muller et al. 2011)

Muller et al. (2011) addressed a question why N_{area} increases in winter. They determined photosynthetic rate and N_{area} in leaves of *Aucuba japonica* plants growing under three light regimes. From nitrogen dependence of daily carbon gain, they calculated optimal N_{area} that maximizes nitrogen use efficiency of daily carbon gain (N_{optNUE}; B in Fig. 1.9). Both increasing PFD and decreasing temperature increases the N_{optNUE}. There was a strong correlation between the N_{optNUE} and actual N_{area}, which was close to the 1:1 relationship (Fig. 1.11). Sensitivity analyses showed that both temperature and PFD had comparable contribution to the change in the variation in the N_{optNUE}.

Vertical gradient of N_{area} has been discussed with respective to maximizing canopy photosynthesis. As mentioned above, photosynthetic rate is less sensitive to N_{area} at low PFD but increases with increasing N_{area} at high PFD. Therefore canopy photosynthesis is improved when nitrogen is allocated more to leaves that receive higher PFD (Field 1983; Hirose and Werger 1987b). Field (1983) showed that canopy photosynthesis is maximized if nitrogen is allocated such that every leaf satisfies following equation:

$$\lambda = \partial P / \partial N \tag{1.1}$$

where λ is the Lagrange multiplier, P is daily carbon gain and N is N_{area}. When compared at the same N_{area}, $\partial P / \partial N$ is higher in leaves that receive high light (Fig. 1.9d). Therefore N_{area} should be higher in upper leaves. Farquhar (1989) suggested that canopy photosynthesis is maximized if P_{max} of each leaf is proportional to light availability of each leaf. Anten et al. (1995) proved that this relationship is maintained when the initial slope and convexity of the light-response curve is constant across leaves. Hirose and Werger (1987b) calculated optimal

nitrogen distribution among leaves in a canopy of *Solidago altissima*, a perennial herb. The actual nitrogen distribution was significantly different from the inferred optimal distribution, as it was less steep. Similar differences between actual and optimal nitrogen distribution were reported by other researchers (Anten et al. 1995). Anten et al. (2000) compiled data obtained from herbaceous canopies and showed that the slope of the actual nitrogen distribution was almost half of that of the optimal distribution.

It should be noted that optimal nitrogen content to maximize daily NUE (N_{optNUE}) is not necessarily consistent with the optimal nitrogen allocation among leaves to maximize canopy photosynthesis. In N_{optNUE}, the curve of daily CER-N has a tangent from the origin (Fig. 1.9b). Slope of the tangent is different depending on PFD (B in Fig. 1.9b). On the other hand, slope of a tangent of the curve, i.e. $\partial P/\partial N$, is required to be identical among leaves in a canopy that maximizes canopy photosynthesis (C for high light leaves in Fig. 1.9b, d). Nitrogen distribution may be less steep in a canopy in which every leaf has N_{optNUE} than in a canopy that maximizing canopy photosynthesis. Therefore, less steep nitrogen distribution found in actual canopies may be caused by a result of optimal regulation at a leaf level rather than that at a canopy level.

1.4   Trade-Offs in Leaf Morphology

Sun and shade leaves differ from each other in morphological traits as well as in physiological traits. In general, sun leaves are thicker and have higher leaf mass per area than shade leaves. Also in tree canopies, there is a vertical gradient in morphological traits (Ellsworth and Reich 1993; Niinemets 1997; Wright et al. 2006). Figure 1.1 shows gradients of leaf traits in a *Fagus crenata* canopy. Leaf mass per area (LMA) exhibited a large decrease from the top to the bottom. N_{area} can be expressed as a product of LMA and nitrogen concentration per mass (N_{mass}). In tree canopies, gradient of N_{area} is mainly attributed to LMA because N_{mass} is relatively constant or even higher in lower canopies (Fig. 1.1). In herbaceous canopies, in contrast, gradient of N_{area} is mainly ascribed to N_{mass} (Hirose et al. 1988). This difference reflects differences in canopy development. In herbaceous canopies, new leaves are mainly formed at the top of the canopy and light availability for each leaf declines with development of new leaves. New leaves developed as a sun leaf and N_{area} and N_{mass} gradually decrease mainly due to resorption while morphological traits are relatively constant (but LMA generally exhibits small reduction through leaf senescence). In tree canopies, on the other hand, new leaves are produced in each layer and light availability does not change greatly. Leaf thickness and LMA were altered according to the environment where the leaves developed, while N_{mass} is relatively constant (Ellsworth and Reich 1993).

Leaf morphology is an important constraint for P_{max} (Terashima et al. 2001). Large investment of photosynthetic proteins is necessary to achieve high P_{max}. Since all photosynthetic enzymes are involved in chloroplasts, sun leaves need to

have a large number of chloroplasts in the mesophyll cells. CO_2 diffusion in the liquid phase is very slow and chloroplasts distribute near the cell surface. If a leaf increased the number of chloroplasts without thickening the mesophyll layer, some chloroplasts would become separated from the cell surface and any increase in the number of such chloroplasts contributes little to increasing photosynthetic capacity because they do not receive sufficient CO_2 to fix. Therefore, sun leaves are thick in order to arrange all chloroplasts along the mesophyll cell surface. Hence there are strong correlations between photosynthetic capacity and leaf thickness (McClendon 1962; Jurik 1986), between photosynthetic capacity and mesophyll cell surface area (Nobel et al. 1975), and between the internal conductance of CO_2 and chloroplast surface area facing the intercellular space (von Caemmerer and Evans 1991; Evans et al. 1994).

This constraint of P_{max} brings about a trade-off between leaf thickness and leaf area. If biomass is limited for production of a leaf, large leaf area is advantageous for light capture but it inevitably forces small leaf thickness and thus suppresses P_{max}. In fact, leaf area in *Fagus crenata* canopy was greater at lower layers (Fig. 1.1).

Gap formation abruptly increases light availability for understorey plants in a forest. This event is considered indispensable for further growth of tree seedlings and thus for regeneration of forests (Denslow 1987; Naidu and DeLucia 1997; Ryel and Beyschlag 2000). In a mixed temperate forest, gaps are formed throughout a year (Romme and Martin 1982). When irradiance increased in the growing season, plants often showed light acclimation where P_{max} increased even in already expanded leaves (Turnbull et al. 1993; Naidu and DeLucia 1998; Yamashita et al. 2000). Nevertheless, it has been shown that leaf thickness is determined by the irradiance at leaf development, and changes little after leaves have matured (Milthorpe and Newton 1963; Verbelen and De Greef 1979; Sims and Pearcy 1992). Does this imply that leaves do not have to become thick to increase their P_{max}?

Oguchi et al. (2003) found that mature shade leaves of *Chenopodium album*, an annual herb, have vacant space along the mesophyll surface which is not occupied by chloroplasts (Fig. 1.12). When the shade leaves were exposed to high irradiance, chloroplast volume increased to fill the space and P_{max} increased without an increase in leaf thickness. However, these leaves had vacant space and consequently were thicker than the minimum required to arrange all chloroplasts to fill the mesophyll cell surface.

Oguchi et al. (2005, 2006) investigated leaf anatomy of various deciduous tree species in a growth cabinet (Oguchi et al. 2005) and in the field where an artificial gap was formed (Oguchi et al. 2006). They found that the response of existing leaves to increasing PFD was different among species. Shade leaves of *Betula ermanii*, *Kalopanax pictus*, *Magnolia obovata*, and *Quercus crispula* had the vacant space in mesophyll cells and increased chloroplast volume after exposure to high light, similar to the results on *C. album* (Fig. 1.13). Three *Acer* species, *A. rufinerve*, *A. mono*, and *A. japonicum* extended not only chloroplast volume but also mesophyll cell surface after exposure to high light, suggesting that *Acer* species have
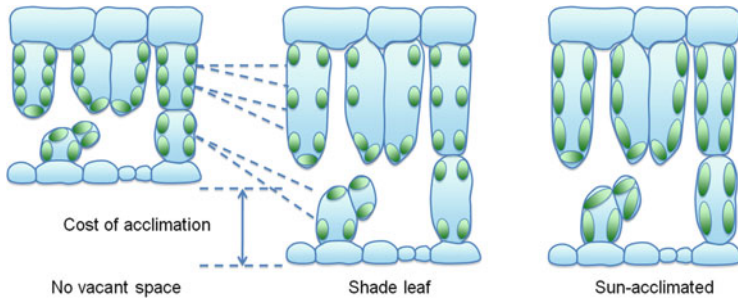
Fig. 1.12 Diagram of anatomical acclimation in shade leaves that are exposed to a sunny condition
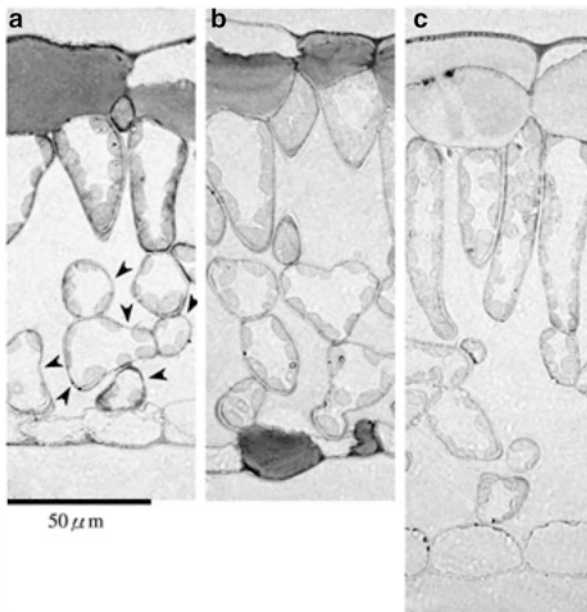

Fig. 1.13 Leaf anatomy of *Betula ermanii*. (**a**) Low-light grown leaf, (**b**) low-light grown leaf after transfer to high light, and (**c**) high-light grown leaf. *Arrows*: vacant space (Redrawn from Oguchi et al. 2005)

plasticity in leaf anatomy even after full expansion (Fig. 1.14). On the other hand, *F. crenata* had little mesophyll cell surface unoccupied by chloroplasts and leaf anatomy was not changed after exposure to high light (Fig. 1.15). Consequently, it did not increase P_{max}. These results suggest that light acclimation potential is primarily determined by the availability of unoccupied cell surface into which chloroplasts expand, as well as by the plasticity of the mesophyll that allows an increase in its surface area.