

論文 (Original article)

Coordination between leaf water relations and leaf gas exchange
in two ecotypes of *Fagus crenata* treeAkira UEMURA^{1)*}, Hisanori HARAYAMA¹⁾ and Atsushi ISHIDA²⁾

Abstract

Fagus crenata Blume is a dominant species in late-successional, cool-temperate forests of Japan. *F. crenata* has two ecotypes in Japan, one in the Japan Sea region and the other in the Pacific Ocean region. The trees at Nakoso in the Pacific Ocean region suffer higher evaporative demand during the growing season than do those at Appi in the Japan Sea region. This study aimed to determine how leaf morphology and leaf physiology are coordinated to cope with air drought conditions. We investigated intra-crown leaf plasticity for adult trees growing in both regions. The specific hypothesis was that the sun leaves of *F. crenata* at Nakoso would have a lower osmotic potential and a larger vascular structure within their leaves than those at Appi and, as a result, would have a relatively higher leaf mass per area (LMA) and lower carbon assimilation capacity. Relative to trees at Appi, the sun leaves at Nakoso had a thicker lamina, a larger bundle sheath within the lamina, and a smaller compartment area circumscribed by a bundle sheath extension. Even though LMA in the sun leaves at Nakoso was 1.74 times larger than that at Appi, the area-based photosynthetic rates did not differ between sites. Leaf morphological acclimation to high evaporative conditions at Nakoso included reduced mass-based nitrogen (N) content and the N/C ratio within the lamina as well as reduced mass- and nitrogen-based assimilation rates. Our study indicates that drought tolerance at the leaf level leads to variations in leaf water relations and in leaf morphology. This suggests a trade-off or coordination between leaf water relations and leaf morphology or leaf gas exchange for drought tolerance.

Key words: intraspecific difference, LMA, Japanese beech, water relations, photosynthesis

Introduction

Plant leaves have morphological and physiological plasticity to cope with unsuitable environments. During photosynthesis, leaves lose water and, by necessity, try to acquire carbon while reducing water consumption. As such, leaf and canopy morphological traits are associated with plant water use. For example, xeric sites favor high leaf mass per area (LMA) (Abrams 1994, Abrams and Mostoller 1995, Mediavilla et al. 2001, Pita and Pardos 2001, Wright and Westoby 2002). The high LMA results from the acquisition of hard tissue structures formed of thick cell walls and a dense vascular system or mesophyll tissue, which help lengthen leaf lifespan (Wright and Westoby 2002, Ishida et al. 2007) and may help prevent the collapse of xylem conduits under low leaf water potentials (Hacke and Sperry 2001, Ishida et al. 2008). A high LMA consequently results in high protein investment to leaf dry mass (mainly, leaf structure) and a small fraction of nitrogen or nutrients allocated to the photosynthetic apparatus (Hikosaka et al. 1998, Poorter and Evans 1998, Reich et al. 1998, Niinemets 1999, Mediavilla et al. 2001, Pons and Westbeek 2004, Takashima et al. 2004, Ishida et al. 2008). Thus, plants experience a trade-off or coordination between leaf structure

and leaf physiology for drought adaptation (Wright and Westoby 2002, Uemura et al. 2004, Ishida et al. 2008).

Fagus crenata Blume is the most abundant cool-temperate deciduous tree species in Japan. The population is divided into at least two ecotypes: the Pacific Ocean type and the Japan Sea type (Hagiwara 1977). The canopy leaves of the Pacific Ocean type are smaller and thicker (Koike and Maruyama 1998, Tateishi et al. 2010, Ishii et al. 2018) and have photosystem II, with a higher tolerance against strong sunlight (Yamazaki et al. 2007) than those of the Japan Sea type. Despite Japan's narrow geographical area, many plant species in Japan have ecotypes or varieties that are split between the Pacific Ocean and Japan Sea regions, with special adaptations to each. For example, plants have specialized morphologies and physiologies for coping with heavy snow in winter in the Japan Sea region (Kume and Ino 1993, Yamazaki et al. 2007) or with high evaporative demand in summer in the Pacific Ocean region (Uemura et al. 2004, Yamazaki et al. 2007). Although cool-temperate forests in Japan have mesic soil water conditions during the growing season, mature trees may suffer water stress caused by a lack of balance between the water loss from atmospheric evaporative demand and water

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supply from the soil. Tateishi et al. (2010) showed that, based on an investigation of geographical variations in tree water use in *F. crenata*, the interaction between physiological and morphological status can allow trees to cope with diverse environmental conditions across its geographical distribution. However, it is still unknown how drought-adaptive changes in leaf water relations are associated with variations in morphology and gas exchange at the single leaf level. Thus, an ecophysiological comparison of the two *F. crenata* ecotypes should help explain how plants coordinate leaf water relations, leaf morphology, and leaf gas exchange to cope with unfavorable microclimates.

This study aimed to determine how leaf morphology and leaf physiology are coordinated to cope with high evaporative demand using the two *F. crenata* ecotypes in Japan. To do this, we examined many traits related to photosynthesis, water relations, and morphology at the single leaf level and compared the canopy leaves of *F. crenata* adult trees in the Japan Sea and Pacific Ocean regions. We selected the following parameters: (1) leaf morphology and anatomy, including LMA, leaf density, stomata, mesophyll tissue, and vascular structure within leaves; (2) leaf physiology, including leaf nitrogen (*N*) content and mass-, area-, and *N*-based photosynthetic rates; and (3) leaf water relations, including leaf pressure–volume curves and minimum conductance for water vapor in both sun and shade leaves. Although several studies have reported on the geographical clines of *F. crenata* (e.g., Hiura 1998, Hiraoka and Tomaru 2009), few have focused on the plasticity of leaf properties (e.g., Yamazaki et al. 2007, Bayramzadeh et al. 2008). Therefore, we compared individual sun and shade leaves to evaluate differences in the plasticity of leaf properties. Our specific hypothesis was that sun leaves in the Pacific Ocean region would have large vascular structures within the leaves, low osmotic potentials to cope with high evaporative demand, and that these properties would result in high LMA and low mass- and nitrogen-assimilation rates in their canopy leaves. Additionally, we hypothesized that shade leaves, which grow in conditions with low evaporative demand, would be less stressed and, therefore, would not show differences between the two ecotypes.

Materials and methods

Plant materials and study sites

Hagiwara (1977) divided *F. crenata* populations in Japan into two ecotypes: the Pacific Ocean type and the Japan Sea type. In contrast, Fujii et al. (2002) classified *F. crenata* populations in Japan into three clades based on cpDNA variations: the haplotype of clade I is distributed mainly along the Japan Sea, whereas those of clades II and III occur chiefly along the Pacific Ocean. The haplotypes of clade III have the

southernmost distribution of the three clades.

We selected mature *F. crenata* trees growing in the two ecotypes. Trees from the Pacific Ocean type (clade II) were located at Nakoso (36°58'N, 140°36'E, altitude 700 m) in Fukushima Prefecture. Trees from the Japan Sea type (clade I) were located at Appi (40°00'N, 140°56'E, altitude 825 m) in Iwate Prefecture. The distance between the two sites was 338 km. The study site at Appi (Appi site) was located in a mid-cool-temperature forest, whereas the site at Nakoso (Nakoso site) was located in a border area between cool-temperature and warm-temperature forests, suggesting that the Nakoso site is more xeric than the Appi site. The Andosol soil was classified as a moderately moist brown forest soil. Canopy leaves of one mature, adult tree were obtained with a scaffolding tower at each site. The forest at the Nakoso site comprised many winter deciduous tree species, such as *Fagus japonica* Maxim., *Betula grossa* Sieb et Zucc., *Quercus serrata* Thunb., *Quercus mongolica* Fisher var. *grosseserrata*, *Carpinus laxifolia* Blume.; this forest type is referred to as a Pacific Ocean type beech forest. The forest at the Appi site consisted mostly of *F. crenata* trees and a small number of winter deciduous tree species, such as *Tilia japonica* Simon. and *Q. mongolica*; this type of forest is referred to as a Japan Sea type beech forest. The top canopy heights of the trees examined at the Nakoso and Appi sites were 14 and 18 m high, respectively, and their ages were approximately 100 and 70 years, respectively. At both sites, *F. crenata* trees opened new leaves at the beginning of May, which fell at the end of October. Exposed canopy leaves at the uppermost crown were collected as sun leaves and the lowermost crown leaves were collected as shade leaves. In August, the amount of daily photosynthetic photon flux (PPF) at the lowermost crown was approximately 7% of that at the uppermost crown. Investigations of morphological and physiological properties were conducted in August 2005, in the middle of the growing season.

The Nakoso site had little snow cover during winter. At the Appi site, the maximum snow depth was approximately 3 m and snow covered the forest floor from November to May. Microclimatological data, photosynthetic photon flux (PPF, $\mu\text{mol m}^{-2} \text{s}^{-1}$), air temperature (°C), air relative humidity (%), and precipitation (mm) at the top of the canopies were recorded at 10-min intervals with a data logger (MES-UL120, Koito Co., Tokyo, Japan) during the growing season (from April to November). PPF values above the canopies were measured with a solar sensor, LI190-SB (LI-COR Inc., Lincoln, NE, USA) at the Appi site and with an IKS-25 (Koito Co.) at the Nakoso site. Air temperature and relative humidity were measured with HMP45D and HMP45A thin-film capacitance sensors (Vaisala KK, Tokyo, Japan), respectively. Precipitation was measured using a tipping bucket rain gage (Sato-keiryoubi,

Tokyo, Japan) at the top of the canopy. The annual mean temperature was 2.4°C higher at the Nakoso site than at the Appi site (Table 1). The warmth index was 17.1 higher at the Nakoso site than at the Appi site.

Leaf gas exchange

Net photosynthetic rates (A) and stomatal conductance were measured with an open, portable measurement system (LI-6400, LI-COR) equipped with a leaf chamber with a chlorophyll fluorescence meter (i.e., fluorometer cuvette, model LI-6400-40). Five randomly selected, fully expanded, intact sun and shade leaves were measured on a sunny day in August between 08:00 and 11:00. The maximum photosynthetic capacity (A_{\max}) was measured under 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 370 $\mu\text{mol mol}^{-1}$ CO_2 concentration in the inlet gas stream, and at 25°C leaf temperature and was expressed as an area-based A_{\max} , mass-based A_{\max} , and N-based A_{\max} (PNUE) values. The parameters $V_{c_{\max}}$ and J_{\max} (Farquhar et al. 1980) were estimated from assimilation rates plotted against the intercellular CO_2 concentration (C_i) (A/C_i curve) under 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF and 25°C leaf temperature. CO_2 concentrations in the chamber were adjusted to 50, 100, 150, 200, 300, 370, 500, 700, 1500, 1800, and 2000 $\mu\text{mol mol}^{-1}$. The values of $V_{c_{\max}}$ and J_{\max} are the “apparent” values for the maximum rate of ribulose 1,5 bisphosphate (RuBP) carboxylation and the maximum rate of electron transport driving the regeneration of RuBP, respectively. $V_{c_{\max}}$ was calculated by fitting the initial slope of the A/C_i curve (under $C_i < 300 \mu\text{mol mol}^{-1}$). I^* , K_c , and K_o were assumed to be 41.9, 283, and 195 mmol mol^{-1} , respectively, according to von Caemmerer et al. (1994). J_{\max} was calculated by fitting a near-plateau of the A/C_i curve (under $C_i > 1600 \mu\text{mol mol}^{-1}$).

Leaf water relations

To measure leaf water relations, pressure-volume (P-V)

curves were constructed with measurements from a pressure chamber (Model-3000, SoilMoisture Equipment Co., Santa Barbara, CA, USA). Shoots were collected from the tree canopies and the cut ends were immediately recut under distilled water, covered with a plastic bag, and transported to our laboratory. The shoots were fully rehydrated under dim light and moist conditions overnight. Osmotic potentials at full turgor (ψ_o^{sat}), leaf water potentials at the turgor loss point (ψ_w^{tlp}), the relative water content at the turgor loss point, the bulk modulus of elasticity in cell walls, and the symplastic water volume in leaves at full turgor (V_s) were calculated from the P-V curves. The maximum bulk modulus of elasticity in the leaf cell walls was determined by linear regression between the turgor potentials and free water contents at the points of the steepest slope. The volume of leaf water (V_l) was calculated as

$$V_l = (\text{water-saturated leaf mass}) - (\text{leaf dry mass}) \quad (1)$$

The number of osmoles in symplastic water within the leaf (N) was calculated as

$$N = \frac{-\psi_o^{\text{sat}} V_s}{R (T + 273.15)}, \quad (2)$$

where R is the gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$) and T is air temperature (Tyree and Hammel 1972).

To examine cuticle development on the leaf surface or the potential of stomatal closure, the minimum conductance for water vapor (g_{\min} : m s^{-1}) was calculated from the rate of water loss in detached leaves. Measurements were conducted under dim light ($<10 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF), at 28°C air temperature, and at 40%–45% relative humidity in a laboratory. The leaf was cut from the shoot, the petiole end was sealed with petroleum jelly, and then the leaf was placed on a balance with 0.00001 g accuracy. The fresh leaves were weighed at 1-min intervals for 10 min. The g_{\min} values were expressed on a water vapor

Table 1. Seasonal changes in microclimate. The monthly mean air temperature, cumulative photosynthetic photon flux (PPF), and precipitation at the Appi and Nakoso sites are presented.

	April	May	June	July	August	September	October	November
Air temperature (°C)								
Appi	4.7	9.3	14	16.9	18	14.3	8.3	2.6
Nakoso	7.2	11.4	16.5	20	20.4	16.8	10.4	5.3
Cumulated PPF ($\text{mol m}^{-2} \text{ month}^{-1}$)								
Appi	1059	1103	1021	855	850	764	572	399
Nakoso	861	918	858	867	783	639	497	416
Precipitation (mm month^{-1})								
Appi	47	55	96	191	198	128	138	81
Nakoso	83	110	96	231	221	220	240	90

The mean values for 5 years (2001–2005) are shown.

concentration and total leaf area basis (2× the projected leaf area), according to Kerstiens (1996).

Leaf morphological and biochemical properties

To estimate the individual leaf area, we collected 30 sun leaves and 30 shade leaves at the Nakoso and Appi sites, respectively. We used the allometric formula as follows:

$$LA = 0.00621 \times L \times W \quad (r^2 = 0.99) \quad (3)$$

where LA is the leaf area (cm^2), L is the lamina length (cm), and W is the lamina width (cm). Individual leaf areas were obtained with a digital scanner and image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA). Only the regression line (equation 3) was used to estimate individual leaf areas, because there was no significant difference between the Appi and Nakoso sites and between the sun and shade leaves in the relationship. For sun and shade leaves at both sites, the area of 100 leaves attached to the branch was estimated from this regression.

Leaf morphological and biochemical properties were investigated in fully expanded leaves collected in August. LMA (g m^{-2}). Leaf gas exchange was measured and then lamina nitrogen and carbon contents were examined in those same leaves with an N-C analyzer (Sumigraph NC-900, Sumika-Bunseki Center, Osaka, Japan). Additionally, the lamina

thickness (μm), leaf density (g cm^{-3}), stomatal density, stomatal pore area, and compartment area of lamina circumscribed by bundle sheath (compartment area, mm^2) were examined (the number of measurements is shown in Table 2). Stomatal observations were conducted by obtaining a replica of each leaf surface with a celluloid plate. The stomata of *F. crenata* are located on the abaxial side (hypostomatous) only. Stomatal density (n mm^{-2}) was calculated from observations in a 0.25 mm^2 field of view, avoiding veins, with a microscope, for five sun and shade leaves. Stomatal pore area (μm^2) was calculated by measuring five stomata per leaf; the distance between the outside edges of the cuticular ledges that overarched the pore was measured. Leaf cross sections were used to assess the vascular structure within the lamina. The lengths of the palisade and spongy parenchyma and the upper and lower epidermis, including the cuticle layers, were measured using fresh leaf cross sections and a microscope. To measure the lamina vascular system, the compartment area circumscribed by the bundle sheath extension was measured from digital photographs of five leaves taken with a stereomicroscope.

Results

Seasonal changes in microclimate in 2005 are shown in Fig. 1. Although there was no large difference between both study sites for the cumulated PPFs during the growing season, the daily maximum values of air vapor pressure deficit (VPD)

Table 2. Mean values \pm 1 S.D. for morphological, anatomical, and biochemical parameters in the sun and shade leaves at the Appi and Nakoso sites.

	Units	<i>n</i>	Appi		Nakoso	
			Sun	Shade	Sun	Shade
Morphological properties						
Leaf area	cm ²	100	26.2 ± 7.0 a	42 ± 13.0 b	11.4 ± 3.9 c	18.7 ± 7.6 d
Leaf mass per area	g m ⁻²	10	61.6 ± 7.9 b	37.8 ± 5.8 a	107.4 ± 5.6 c	30.9 ± 2.9 a
Leaf density	g cm ⁻³	10	0.392 ± 0.030 a	0.307 ± 0.053 b	0.473 ± 0.017 c	0.258 ± 0.022 d
Stomatal density	n mm ⁻²	5	200 ± 40 b	128 ± 16 a	248 ± 23 c	126 ± 19 a
Stomatal pore area	µm ²	5	63 ± 8 a	66 ± 12 a	56 ± 7 a	59 ± 5 a
Compartment area	mm ²		0.084 ± 0.0223 a	0.146 ± 0.0110 b	0.035 ± 0.0033 c	0.097 ± 0.0141 a
Anatomical properties						
Total thickness	µm	5	151.3 ± 15.8 b	111.2 ± 8.7 a	207.4 ± 15.7 c	97.1 ± 7.2 a
Upper epidermis	µm	5	11.2 ± 0.9 a	10.3 ± 0.6 a	14.6 ± 1.8 b	8.1 ± 1.0 c
Palisade mesophyll	µm	5	50.4 ± 5.2 b	30.9 ± 4.2 a	94.6 ± 5.9 c	24.8 ± 2.7 a
Spongy mesophyll	µm	5	79.5 ± 9.9 a	61.6 ± 6.1 b	87.1 ± 10.0 a	57 ± 4.1 b
Lower epidermis	µm	5	10.1 ± 0.8 a	8.4 ± 0.8 b	11.1 ± 0.7 a	7.1 ± 0.3 c
Biochemical properties						
Mass-based nitrogen	%	10	2.57 ± 0.06 a	2.81 ± 0.09 b	2.11 ± 0.09 c	2.6 ± 0.06 a
Area-based nitrogen	g m ⁻²	10	1.58 ± 0.21 a	1.06 ± 0.17 b	2.27 ± 0.13 c	0.79 ± 0.06 d
Nitrogen per leaf	g leaf ⁻¹	10	5.05 ± 1.23 a	4.47 ± 0.85 ab	3.78 ± 0.66 b	1.87 ± 0.59 c
Nitrogen/carbon ratio		10	0.053 ± 0.0011 a	0.058 ± 0.0021 b	0.041 ± 0.0017 c	0.053 ± 0.0012 a
Lamina absorbance (400–700)		7	0.901 ± 0.003 a	0.87 ± 0.007 b	0.902 ± 0.005 a	0.876 ± 0.007 b

The different letters for each parameter show significant differences ($P < 0.05$) in a Tukey's HSD test.

Compartment area refers to the lamina area surrounded by the bundle sheath.

were higher at the Nakoso site than at the Appi site throughout the growing season. We speculate that the difference between sun leaves and shade leaves on the leaf-to-air VPD was even larger because of solar radiation. The daily maximum VPD at the Nakoso site often exceeds 1.5 kPa. For *Fagus sylvatica* L., 1.5 kPa is the threshold for stomatal closure (Lendzion and Leuschner 2008, de Carcer et al. 2017). The total precipitation during the growing season at the Nakoso and Appi sites was 1291 and 932 mm, respectively. Throughout the growing season, the pre-dawn leaf water potential at the measured height was high enough, suggesting that the trees at both sites have little soil water stress. The maximum no-rain period was about 1 week.

Individual leaf area and lamina thickness significantly

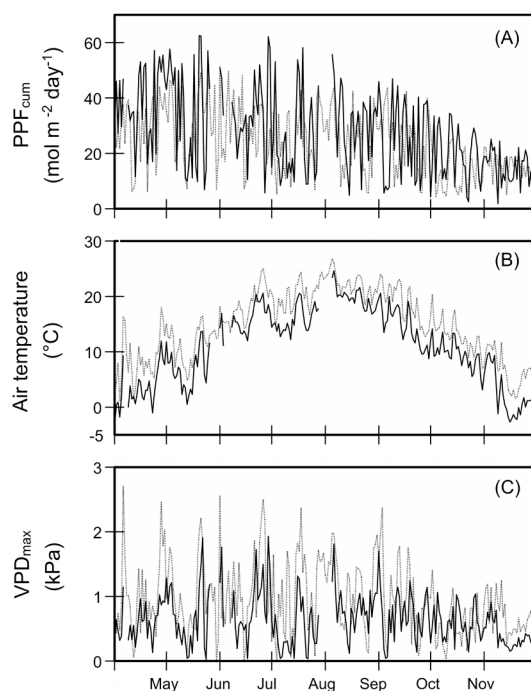


Fig. 1. Seasonal courses of the daily total photosynthetic photon flux (A, PPF_{cum}), mean air temperature (B), and daily maximum air vapor pressure deficit (C, VPD_{max}) in 2005. Data were measured at the canopy tops. The thick and dotted lines represent values at Appi and Nakoso, respectively.

differed between the sun leaves (Na-sun) at Nakoso and those at Appi (Ap-sun). The leaf area in Na-sun was 44% of that in Ap-sun, and the lamina thickness in Na-sun was 137% of that in Ap-sun (Table 2). The LMA in Na-sun was 1.74 times larger than that in Ap-sun, resulting from the thick lamina and high leaf density in Na-sun. The difference in lamina thickness was mainly due to the difference in the thickness of the palisade mesophyll layer, rather than the thickness of the spongy mesophyll layer. The two layers of palisade parenchyma in Na-sun were more expanded than in Ap-sun (Fig. 2). As a result, the palisade tissue length/spongy tissue length ratio was higher in Na-sun than in Ap-sun. The upper epidermis was also thicker in Na-sun than in Ap-sun, and the bundle sheaths were larger in Na-sun than in Ap-sun (Fig. 2 and Table 2). The compartment area circumscribed by the bundle sheath extension (i.e., compartment area) was smaller in Na-sun than in Ap-sun. Stomatal density was 1.24 times larger in Na-sun than in Ap-sun, but no significant difference in pore area was found between Na-sun and Ap-sun. Most morphological parameters showed no significant difference between the shade leaves at Nakoso (Na-shade) and those at Appi (Ap-shade), except that the individual leaf area in Na-shade was 45% of that in Ap-shade and the leaf density in Na-shade was 85% of that in Ap-shade.

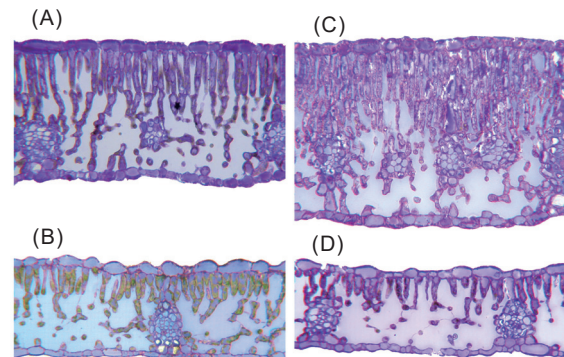


Fig. 2. Micrographs of transverse sections of the lamina of (A) sun leaves at Appi, (B) shade leaves at Appi, (C) sun leaves at Nakoso, and (D) shade leaves at Nakoso sites. The bar indicates 0.1 mm.

Table 3. Mean values ± 1 S.D. for the water relation parameters in the sun and shade leaves at the Appi and Nakoso sites.

	Units	Appi		Nakoso	
		Sun	Shade	Sun	Shade
Osmotic potential at full turgor	MPa	-1.89 ± 0.02 a	-1.66 ± 0.00 b	-2.12 ± 0.00 c	-1.81 ± 0.13 ab
Water potential at turgor loss point	MPa	-2.07 ± 0.03 ab	-1.85 ± 0.05 a	-2.68 ± 0.04 c	-2.17 ± 0.14 b
Relative water content at turgor loss point	MPa	0.95 ± 0.01 a	0.92 ± 0.01 ab	0.92 ± 0.01 ab	0.897 ± 0.02 b
Maximum module of elasticity in the cell wall	MPa	29.3 ± 4.4 a	34.9 ± 9.8 a	27.9 ± 1.8 a	27.9 ± 4.6 a
Dry mass-based V_t	kg H ₂ O kg ⁻¹	1.16 ± 0.04 a	1.55 ± 0.05 b	1.06 ± 0.05 a	1.46 ± 0.14 b
V_s/V_t ratio		0.59 ± 0.04 a	0.73 ± 0.03 ab	0.36 ± 0.06 c	0.61 ± 0.10 ab
N/V_t ratio	Osmoles kg H ₂ O ⁻¹	0.46 ± 0.04 a	0.49 ± 0.02 a	0.34 ± 0.03 b	0.45 ± 0.05 a

Different letters in each parameter show significant differences ($P < 0.05$) in a Tukey's HSD test.

V_t = total water volume in leaves at full turgor; V_s = symplastic water volume at full turgor; N = number of osmoles in the cells.

Na-sun had the lowest N on a mass basis but the highest N on an area basis among the four types of leaves because of the highest LMA (Table 2). The mass-based N values were higher in the shade leaves than in the sun leaves, whereas the area-based N values were lower in the shade leaves than in the sun leaves at both sites. Na-sun had the lowest N/C ratio among the four types of leaves.

The minimum conductance for water vapor of leaves (g_{\min}) was the highest in Ap-sun among the four types of leaves (Fig. 3), indicating undeveloped cuticles or loose stomatal closure. The values of g_{\min} ranged from 6.4 to $20.1 \text{ m s}^{-1} \times 10^5$.

In leaf water relations, Na-sun had the lowest osmotic potential at full turgor (ψ_o^{sat}) and leaf water potential at turgor loss point (ψ_w^{tlp}) among the four types of leaves (Table 3), indicating that Na-sun can have more negative leaf water

potentials during daytime while maintaining turgor pressure. There was no difference in ψ_o^{sat} or ψ_w^{tlp} between Ap-sun and Na-shade, indicating that the canopy leaves at Appi were not experiencing severe drought. The values of ψ_w^{tlp} were smaller for Na-shade than for Ap-shade. There was no difference for the maximum modulus of elasticity among the four types of leaves. Although there was no significant difference in total water volume in leaves at full turgor (V_t) per unit dry mass between Na-sun and Ap-sun, the ratio of symplastic water volume to total water volume within leaves (V_s/V_t ratio) was lower in Na-sun than in Ap-sun. The ratio of the number of osmoles to total water volume (N/V_t ratio) was lower in Na-sun than in Ap-sun. There were no significant differences in the dry mass-based V_t , V_s/V_t ratio, or N/V_t ratio between Na-shade and Ap-shade.

The leaf area-based assimilation capacity excluding the effect of stomatal conductance was evaluated from the $V_{c_{\max}}$ and J_{\max} parameters. There was no difference for the area-based $V_{c_{\max}}$ and J_{\max} between Na-sun and Ap-sun (Fig. 4), but the mass-based $V_{c_{\max}}$ and J_{\max} were significantly higher in Ap-sun than in Na-sun (data not shown), indicating a high investment

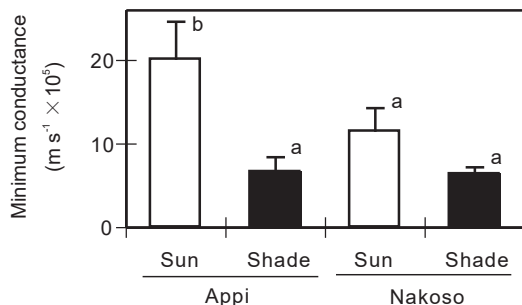


Fig. 3. Minimum conductance for water vapor in detached leaves ($n = 5$). Bars show ± 1 S.D. Different letters show significant differences ($P < 0.05$) in a Tukey's HSD test.

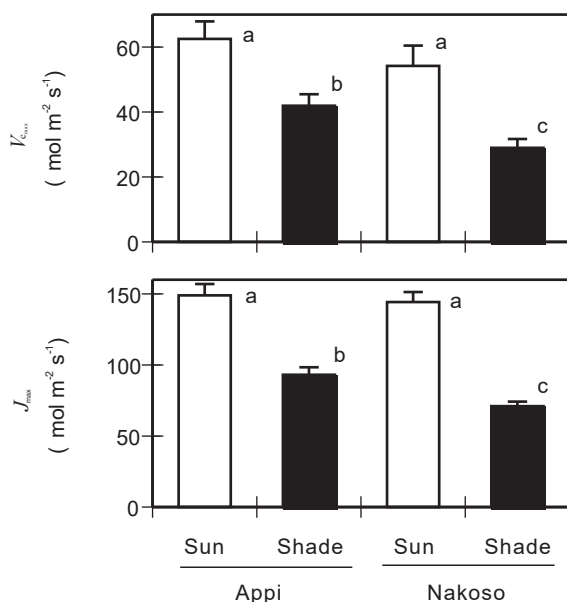


Fig. 4. Maximum rates of (top panel) RuBP carboxylation ($V_{c_{\max}}$) and (bottom panel) electron transport driving the regeneration of RuBP (J_{\max}) ($n = 5$). Bars show ± 1 S.D. Different letters show the significant differences ($P < 0.05$) in a Tukey's HSD test.

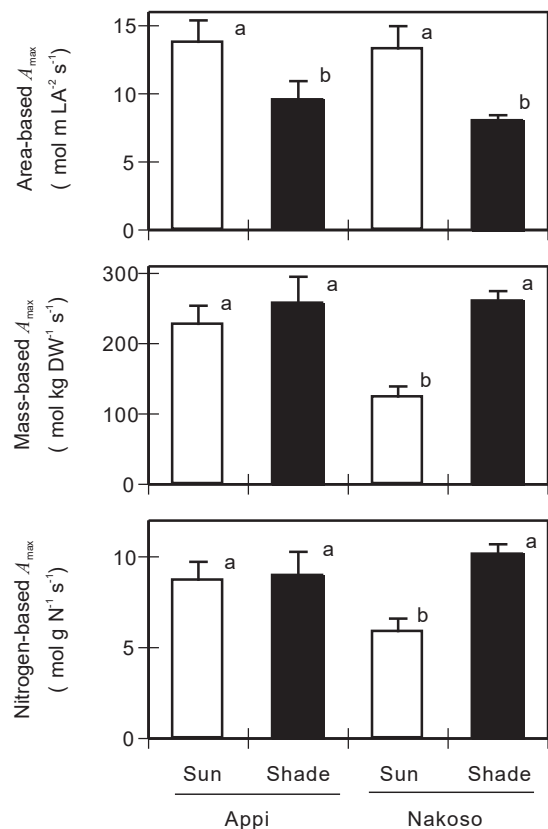


Fig. 5. Daily maximum net photosynthetic rates (A_{\max}) on a (top panel) leaf area basis, (middle panel) leaf dry mass basis, and (bottom panel) nitrogen basis ($n = 5$). Bars show ± 1 S.D. Different letters show the significant differences ($P < 0.05$) in a Tukey's HSD test.

of resources to the photosynthesis apparatus within lamina in Ap-sun. The area-based $V_{c_{\max}}$ and J_{\max} values were higher in the sun leaves than in the shade leaves in both sites.

Net photosynthetic rates (A_{\max}), including the effect of stomatal conductance, were measured under $370 \mu\text{mol mol}^{-1} \text{CO}_2$ (Fig. 5). The differences for area-based A_{\max} among the four types of leaves were similar to those of $V_{c_{\max}}$ and J_{\max} . Although the lamina thickness was higher in Na-sun than in Ap-sun, no difference in the area-based A_{\max} between Na-sun and Ap-sun was found. The mass-based A_{\max} and nitrogen-based A_{\max} (PNUE) were the lowest in Na-sun among the four types of leaves.

The daily maximum stomatal conductance was three times higher in the sun leaves than in the shade leaves, but there was no difference in the stomatal conductance between the sites (Fig. 6).

Discussion

Generally, intra-crown leaf plasticity was higher for trees in the Pacific Ocean than in the Japan Sea genetic lineages. The top canopy leaves at Nakoso (the Pacific Ocean region) suffered from a higher evaporative demand than those at Appi (the Japan Sea region). Nevertheless, precipitation during the growing season was higher at Nakoso than at Appi. For leaf water relations and morphology, there were differences in some properties in the shade leaves, but the differences were more pronounced in the sun leaves, which grow and operate in a harsher environment. In European beech, genotypes with a low-rainfall origin (less than 900 mm (Peuke et al. 2002)) are known to possess more drought-adaptive leaf water relations than genotypes with a high-rainfall origin (Tognetti et al. 1995, Peuke et al. 2002). Our study suggests that Japanese beech is more sensitive to drought than European beech. In contrast, Ishii et al. (2018) showed that intra-crown leaf plasticity is lower for trees in the Pacific than in Japan Sea genetic lineages of *F. crenata*. More investigations are needed to evaluate

adaptability to microenvironments in *F. crenata* genotypes, because tree age (Niinemets 2006, Poorter et al. 2009, Lapok et al. 2017) and height (Ambrose et al. 2009, Zhang et al. 2012, Coble and Cavaleri 2017, Azuma et al. 2019) also affect leaf properties such as LMA.

Our observations showed that the leaf area-based photosynthetic rate in Na-sun was the lowest, regardless of the fact that LMA was the largest among the four leaf types. Nitrogen (N) is a limited resource, so an investment of N in the cell wall reduces its distribution to other areas, such as the photosynthetic apparatus, that is, there is a trade-off between N allocation to photosynthesis and to structural tissues (Onoda et al. 2004, Takashima et al. 2004). The high LMA and low N/C ratio in Na-sun resulted from a thick lamina, a thick cell wall, and dense and large vascular systems within the lamina. A dense vascular system in Na-sun would improve water permeability. For *F. sylvatica* in Italy, Bussotti et al. (2005) showed that trees at the southernmost area, where more xeric conditions exist, have higher LMA and lower N/C ratios than those in the more northern area, where more mesic conditions exist. Negative correlations between wood density and daily minimum water potentials in leaves are widely found across species (Ackerly 2004, Bucci et al. 2004, Santiago et al. 2004, Ishida et al. 2008), probably because hard tissue is needed to avoid implosion of xylem conduits under low leaf water potentials (e.g., Hacke and Sperry 2001). Similarly, high LMA in Na-sun probably helps to tolerate low leaf water potentials in the daytime.

The decrease in leaf osmotic potentials is due to the accumulation of osmoles within leaves (i.e., osmotic adjustment) and/or the decrease in V_s/V_t (e.g., Harayama et al. 2006). In *F. crenata*, no significant difference in total water volume (V_t) per unit dry mass in the sun leaves between both sites was found, and the V_s/V_t and N/V_t ratios were lower in Na-sun than in Ap-sun (Table 3), indicating that a decrease in the symplastic water fraction within leaves in Na-sun contributed to having more negative ψ_o^{sat} without an active accumulation of solutes in cells. An increase in the elasticity of the cell wall can also contribute to turgor maintenance. For example, Li (1998) showed that the elasticity of the cell wall varies among regions with different amounts of precipitation in *Eucalyptus microtheca* F. Muell. In contrast, in our study, no significant difference in the elasticity of the cell wall was found between the sun leaves at the two sites (Table 3). The dense and large vascular system and the small area of the lamina compartment circumscribed by the bundle sheath extension in Na-sun might result in a low V_s/V_t ratio (Bussotti et al. 2005). As the numbers of xylem conduits are positively related to leaf hydraulic conductance (Sack and Frole 2006), the large and dense vascular systems in Na-sun probably cause their leaves to have

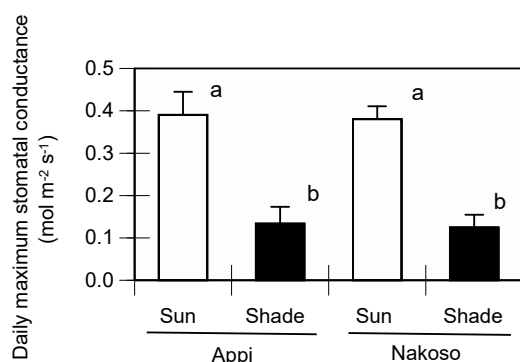


Fig. 6. Daily maximum stomatal conductance ($n = 5$). Bars show ± 1 S.D. Different letters show the significant differences ($P < 0.05$) in a Tukey's HSD test.

high hydraulic conductance.

Leaves under drought conditions usually have smaller and more numerous stomata per unit leaf area than leaves under well-irrigated conditions (Larcher 2003, Bacelar et al. 2004, Pearce et al. 2006). Na-sun had higher stomatal density than Ap-sun, whereas the stomatal density in the shade leaves was the same (Table 2). The high stomatal density in Na-sun is considered a xeromorphic acclimation (Abrams 1990, Abrams 1994, Bussotti et al. 2005). However, our study showed that there was no difference in daily maximum stomatal conductance. The minimum conductance for water vapor (g_{\min}) (cuticle transpiration) indicates the magnitude of the inevitable water loss from the leaf (Burghardt and Riederer 2003). Values of g_{\min} were considerably higher than values reported for *F. sylvatica* ($2.5\text{--}8\text{ m s}^{-1}$) but were within the range of values for winter deciduous tree species (Kerstiens 1996). g_{\min} was significantly lower in Na-sun than in Ap-sun, indicating a reasonable acclimation. Kerstiens (1995) obtained a similar result in *F. sylvatica*. Further studies are needed to determine how minimum conductance is related to drought adaptation.

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太平洋側型ブナと日本海側型ブナ成木樹冠内の葉の可塑性の違い

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要旨

ブナ (*Fagus crenata* Blume) は、日本の冷温帯に優占し、太平洋型と日本海型 2 つのエコタイプを持つ樹種である。太平洋型エコタイプである勿来¹⁾の個体は、日本海型エコタイプである安比²⁾の個体より、高い蒸散要求にさらされる。我々は、ブナの個体内の葉の特性のバリエーションを調べた。この研究の目的は、どのように葉の形態と葉の生理生態学的特性が大気乾燥状態に対応するために関係しているかを明らかにすることである。具体的仮説は、勿来陽葉は、安比陽葉より、低い浸透ポテンシャルそして大きな葉脈構造を持つことである。結果として、勿来陽葉は、高い葉面積当たりの乾燥重量 (LMA) そして低い二酸化炭素同化能力を持つことになる。勿来陽葉は、安比陽葉と比べて厚い葉身、葉身内に大きな維管束鞘、小さな維管束鞘延長部包囲面積を持った。勿来陽葉の LMA は安比陽葉の 1.74 倍だった。勿来陽葉は、日中、葉の膨圧を維持しつつ、より低い葉の水ポテンシャルを持つことができた。勿来の高い蒸散要求状況に対する葉の形態的順化は、結果的に、葉身内の窒素濃度、N/C 率を減らした。そして、乾重当たり、窒素当たりの同化速度を減らした。我々の研究は、葉レベルの乾燥耐性は、葉の水分特性だけでなく形態レベルでも変化を導くことを示す。これは、乾燥耐性に関して葉の水分特性と葉の形態あるいは葉のガス交換の間に連携があることを暗示する。

キーワード：可塑性、LMA、ブナ、水分特性、光合成

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