Relationship among leaf life span, leaf mass per area and leaf nitrogen causes different altitudinal changes in leaf δ^{13}C between deciduous and evergreen species

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Abstract: We examined the variations of stable carbon isotope ratio (δ^{13}C) of leaves of two deciduous broad-leaved species and two evergreen conifer species along an altitudinal gradient in central Japan. The δ^{13}C of the two deciduous species decreased with altitude, except for the upper distribution limit. On the contrary, the two evergreen species showed no clear altitudinal trends of δ^{13}C. The δ^{13}C of the two deciduous species was positively correlated with leaf mass per area (LMA), indicating that the altitudinal variation of δ^{13}C was controlled by LMA. Leaf nitrogen per mass (as a proxy of assimilation capacity, N_{mass}) was negatively correlated with LMA for the two deciduous species, while it was not correlated with LMA for the two evergreen species. Leaf life span of the two deciduous species decreased with altitude, whereas that of the two evergreen species increased. Thus, the two deciduous species had shorter-lived thinner leaves with higher N_{mass} at higher altitudes, and the two evergreen species had longer-lived leaves. These changes contribute to the positive carbon balance at higher altitudes. Therefore, the different altitudinal changes in δ^{13}C between the deciduous and evergreen species are ascribed to the different altitudinal changes in the leaf traits for the carbon balance.

Key words: altitude, foliar δ^{13}C, leaf life span, leaf mass per area, leaf nitrogen, stable carbon isotope ratio.

Introduction

A stable carbon isotope ratio (δ^{13}C) of plant tissues is a clue to understand environmental conditions for the plants because δ^{13}C is associated with the ratio of CO_2 partial pressure within leaves (P_i) to CO_2 partial pressure outside leaves (P_a) (Farquhar 1989). Several experimental studies have confirmed the linear relationship between carbon discrimination and P_i/P_a (see Brugnoli and Farquhar 2000 for review). This ratio (P_i/P_a) is determined by the balance between CO_2 demand and CO_2 diffusive supply within leaves through stomata. CO_2 demand relates to photosynthetic-related factors such as efficiency of CO_2 uptake (Körner 1999), light intensity, and leaf nitrogen (Zimmerman and Ehleringer 1990; Pearcy and Pfitsch 1991; Cordell et al. 1999; Duursma and Marshall 2006). On the contrary, CO_2 supply relates to stomatal density (Hultine and Marshall 2000), stomatal conductance (Meinzer et al. 1992; Hanba et al. 1997), and mesophyll thickness (Terashima et al. 2001). For example, leaf water potential and stomatal conductance of plants often decrease with increasing water stress, and stomatal closure reduces water loss from plants (Granier and Bréda 1996; Kallarackal and Somen 1997; Miller et al. 1998; Oren et al. 1998; Schoettle and Rochelle 2000; Panek and Goldstein 2001), which decreases the CO_2 partial pressure within leaves. Although plants tend to preferentially use $^{12}CO_2$ rather than isotopically heavier $^{13}CO_2$ during carbon assimilation, plants have to assimilate more $^{13}CO_2$ than usual under CO_2-limited conditions due to water stress. Therefore, environmental conditions affect δ^{13}C of plants through CO_2 supply to leaves (Lauteri et al. 1997; Moore et al. 1999; Warren et al. 2001; Van de Water et al. 2002; Ferrio et al. 2003).

Environmental conditions largely change with altitude. In general, the amount of precipitation is greater at higher altitudes, associated with lower air temperature and lower atmospheric pressure. Several studies have shown that δ^{13}C of leaves increases with altitude (Körner et al. 1988, 1991; Vitousek et al. 1990; Marshall and Zhang 1994; Cordell et al. 1999; Hultine and Marshall 2000; Kogami et al. 2001). The causal factor for the increase of δ^{13}C at higher altitudes is the decrease of the P_i/P_a (Körner et al. 1988). Vitousek et al. (1990) showed that the increase of δ^{13}C with altitude was ascribed to the increase of leaf mass per area (LMA). The increase of LMA lengthens the internal diffusion pathway to chloroplasts, which decreases the CO_2 supply, which in turn increases δ^{13}C (Sparks and Ehleringer 1997; Hanba et al. 1999; Niimenets et al. 1999; Takahashi and Mikami 2006). Thus, it is suggested that altitudinal changes in δ^{13}C are strongly regulated by LMA. However, LMA of all plant species does not always increase with altitude (cf. Kudo 1995). Therefore, altitudinal variation of δ^{13}C cannot be explained by LMA alone.

LMA can be considered as an index of leaf construction cost per unit leaf area. LMA changes with altitude or growth period, and this change is often associated with changes of leaf life span and assimilation capacity (Kudo 1999). In deciduous species, both leaf life span and LMA tend to decrease with decreasing growth period. On the contrary, both leaf life span and LMA of evergreen species tend to increase with decreasing growth period (Geeske et al. 1994; Kikuzawa and Kudo 1995). These opposite changes of the plasticity between the deciduous and evergreen species have been suggested to be linked to the maintenance of carbon balance. Short-lived thinner leaves of deciduous species tend to have higher assimilation capacity (Reich et al. 1991, 1992), which compensates for the short growth period at higher altitude. On the contrary, evergreen species need to enhance LMA (i.e., mechanical stiffness) to increase leaf life span, but the increase of leaf life span results in larger construction cost of leaves at high altitudes. If $\delta^{13}C$ of leaves is strongly controlled by LMA, it is expected that $\delta^{13}C$ values of deciduous species decrease and those of evergreen species increase with altitude. However, so far no studies have compared altitudinal changes of $\delta^{13}C$ between evergreen and deciduous species from the viewpoint of the functional relationship among leaf life span, LMA and assimilation capacity.

In this study, we examined altitudinal changes of $\delta^{13}C$ of four representative tree species (two deciduous broad-leaved species and two evergreen conifers) in the subalpine zone in central Japan. The objective of this study was to examine whether deciduous and evergreen species show different altitudinal changes in $\delta^{13}C$ through the functional relationship among LMA, leaf life span and assimilation capacity.

Study site

This study was performed in the subalpine forest zone on the east slope of Mount Norikura (36˚06'N, 137˚33'E, 3026 m above sea level) in central Japan. Mean annual temperature recorded at Nagawa Weather Station (1068 m a.s.l., approximately 12 km in horizontal distance from the summit) was 8.4˚C in 2003. Mean monthly temperatures in the coldest month of January and the hottest month of August were –4.2 and 20.3˚C, respectively. Mean annual precipitation was 2206 mm.

Three major vegetation zones can be recognized between 800 m a.s.l. and the summit on Mount Norikura, i.e., a montane deciduous broad-leaved forest zone between 800 m and 1600 m a.s.l., a subalpine coniferous forest zone between 1600 m and 2500 m a.s.l. and an alpine dwarf pine (*Pinus pumila* Regel) scrub zone between 2500 m and 3000 m a.s.l. near the summit (Miyajima et al. 2007). The timberline is at about 2500 m a.s.l. on the east slope of Mount Norikura (Takahashi 2003).

Dominant tree species are the evergreen conifers *Abies veitchii* Lindl. and *Tsuga diversifolia* Mast. between 1600 m and 2000 m a.s.l., and the evergreen conifer *Abies mariesii* Mast. and deciduous broad-leaved *Betula ermanii* Cham., *Sorbus commixta* Hedl. and *Sorbus matsumurana* Koehne between 2200 m and 2500 m a.s.l. Anthropogenic effects on vegetation were negligible from 1600 m a.s.l. to the summit. Further details on vegetation and stand structure along this altitudinal gradient are given in Miyajima and Takahashi (2007) and Miyajima et al. (2007). Plant nomenclature followed the Editorial Board of Flora of Nagano Prefecture (1997).

Materials and methods

In this study, the four dominant species in the subalpine zone were examined along the altitudinal gradient (1600 m to 2500 m a.s.l.), i.e., two evergreen conifers (*Abies mariesii* and *A. veitchii*) and two deciduous broad-leaved species (*Betula ermanii* and *Sorbus commixta*). According to Miyajima et al. (2007) and this study, altitudinal distribution ranges of species were as follows; *B. ermanii*: 1600 m to 2500 m a.s.l., *S. commixta*: 1600 m to 2200 m a.s.l., *A. veitchii*: 1600 m to 2300 m a.s.l., *A. mariesii*: 1900 to 2500 m a.s.l. (Fig. 1). The two deciduous broad-leaved species are shade-intolerant. Although the two evergreen conifers are shade-tolerant, *A. mariesii* is more shade-tolerant than *A. veitchii* (Kohyama 1983). *S. commixta* is a small tree species, while the three other species are large canopy tree species. Altitudinal changes in water-related leaf physiological traits (stomatal density, stomatal conductance and leaf water potential) of the four species are reported in Takahashi and Miyajima (2008).

We measured $\delta^{13}C$, leaf life span, LMA and leaf nitrogen content per mass (N_{mass}) to investigate their functional relationship with $\delta^{13}C$. N_{mass} is a proxy of assimilation capacity per leaf mass (Reich et al. 1998; Shipley et al. 2005). Measurements were done at three to five sites at 200 m altitudinal intervals between 1600 m and 2400 m a.s.l. for each species in 2003 and 2004 (Fig. 1). Altitudes examined were different among the four species according to their distribution ranges (Fig. 1). Although *A. veitchii* is distributed up to 2300 m a.s.l., the density of this species is low above 2000 m a.s.l. (Miyajima et al. 2007). Therefore, we investigated the leaf traits of *A. veitchii* up to 2000 m a.s.l. (Fig. 1).

At each site, five trees were chosen for each species. We chose similar sized trees that were not shaded by neighboring trees to avoid confounding effects of light environment and ontogeny on leaf traits. Diameter at breast height of trees selected ranged between ca. 3 and 6 cm.

Five to eight sun-exposed leaves of each deciduous broad-leaved species and all needles of five to eight sun-exposed current-year shoots of the evergreen *Abies* species were chosen from the five trees selected per altitude for measurement of LMA, $\delta^{13}C$ and N_{mass}. Leaves were sampled within a day in late August 2003 for *B. ermanii* and two *Abies* species and in early September 2004 for *S. commixta*. Leaf area was measured, using a scanner and a Macintosh computer with the public domain NIH image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Leaves were oven-dried at 80˚C for at least 48 hours and weighed before LMA was calculated for each leaf. Then the leaves were ground to a powder, and $\delta^{13}C$ and N_{mass} were measured using an isotope ratio mass spectrometer (DELTA plus, ThermoQuest Ltd, Thermo Electron Ltd, Yokohama, Japan) and an elemental analyzer (Flash EA1112, ThermoQuest Ltd, Thermo Electron Ltd, Yokohama, Japan). Results are expressed as the deviation (‰) from a standard (Viernna-Pee Dee Belemnite):

$$\delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

where $R = {}^{13}C/{}^{12}C$. The analytical precision was 0.1‰.

Fifteen sun-exposed current-year shoots were selected for the two deciduous broad-leaved species (*B. ermanii* and *S. commixta*) at each altitude in early spring in 2004 to measure leaf life span. Two buds were marked per shoot, and were observed twice a week to determine the date of leaf opening and shedding. Annual shoot elongation in the past can be easily identified for *Abies* species by bud scars (Kimura 1963). Although needles gradually fall with aging of needles, the needle scars leave on the stem. Thus, survival rate of needles for given shoot age can be calculated by the number of survived needles divided by the total number of needles (survived plus fallen needles) of the shoot. Mean needle longevity was determined as the shoot age that retained a majority (> 50%) of their needles. Thus, our data are representative of average needle life span, not maximum needle retention (cf. Reich et al. 1996).

Each leaf parameter ($\delta^{13}C$, LMA and leaf life span) was compared among the altitudes for each species by Tukey HSD multiple comparison test. Relationships of LMA with $\delta^{13}C$ and N_{mass} were examined by Pearson correlation test.

Results

Stable carbon isotope ratios ($\delta^{13}C$) of the two deciduous broad-leaved species

(*B. ermanii* and *S. commixta*) tended to decrease with increasing altitude, but then increased again at the upper distribution limit (Fig. 2). The δ^{13}C of *A. veitchii* was almost constant across the altitudinal gradient. The δ^{13}C of *A. mariesii* was higher at 2400 m a.s.l. than the lower altitudes (Tukey HSD test, $P < 0.05$).

The altitudinal change in leaf mass per area (LMA) of each species was closely related to leaf δ^{13}C (Figs. 2, 3). LMA of the two deciduous broad-leaved species decreased with increasing altitude, but increased again at the highest altitude (2400 m a.s.l. for *B. ermanii* and 2200 m a.s.l. for *S. commixta*, Fig. 3). LMA of the two evergreen *Abies* species was not significantly different among the three examined altitudes (Fig. 3). Foliar δ^{13}C was significantly positively correlated with LMA for the two deciduous broad-leaved species (*B. ermanii* and *S. commixta*) ($P < 0.01$, Fig. 4). Although a significant correlation was recognized in *A. mariesii* ($P < 0.01$, Fig. 4), this correlation was marginally significant if the one point of the largest LMA and leaf δ^{13}C was deleted ($R = 0.502$, $P = 0.067$). The relationship between LMA and leaf δ^{13}C was also marginally significant in *A. veitchii* ($P = 0.066$, Fig. 4). Therefore, intraspecific variation of leaf δ^{13}C was closely and weakly related to LMA in the two deciduous broad-leaved species and the two evergreen conifer species, respectively.

N_{mass} of *B. ermanii* tended to increase with altitude (Fig. 5). Altitudinal change in N_{mass} of *S. commixta* was a reverse trend with that of LMA, i..e, N_{mass} was highest at 2000 m a.s.l. at which LMA was lowest (Figs. 3, 5). N_{mass} of *A. veitchii* increased with altitude, while that of *A. mariesii* decreased (Fig. 5). N_{mass} of the two evergreen conifer species (*A. mariesii* and *A. veitchii*) was not correlated with LMA (Fig. 6). On the contrary, N_{mass} of the two deciduous broad-leaved species (*B. ermanii* and *S. commixta*) was negatively correlated with LMA (Fig. 6), although this relation was marginally significant for *B. ermanii* ($P = 0.077$, Fig. 6). Thus, leaves with smaller LMA have greater N_{mass} (as a proxy of assimilation capacity per leaf mass) in the two deciduous broad-leaved species.

Leaf life span more linearly changed with altitude for the four species, as compared with foliar δ^{13}C, LMA and N_{mass}, and opposite changes were observed between the two deciduous broad-leaved species and the two evergreen conifer species (Fig. 7). Leaf life span of the two deciduous broad-leaved species decreased with increasing altitude (Fig. 7). For example, the leaf life span of *B. ermanii* decreased from 119 d at 1600 m a.s.l. to 86 d at 2400 m a.s.l. The leaf life span of *S. commixta* decreased from 150 d at 1600 m a.s.l. to 117 d at 2200 m a.s.l. On the contrary, the leaf life span of evergreen *A. mariesii* increased from 2338 d (6.41 yr) at 2000 m a.s.l. to 3067 d (8.40 yr) at 2400 m a.s.l. (Fig. 7). The leaf life span of *A. veitchii* also increased from 2016 d (5.52 yr) at 1600 m a.s.l. to 2447 d (6.70 yr) at 2000 m a.s.l.

Discussion

In this study, leaf δ^{13}C changed with altitude except for *Abies veitchii*. The δ^{13}C of the four species was positively correlated with LMA, although this relationship was relatively weak in the two evergreen conifers. Large LMA would lengthen the internal diffusion pathway to chloroplasts, which increases the resistance and thus decreases the CO_2 supply to the site of carboxylation. Therefore, it is suggested that the variation of leaf δ^{13}C is controlled by that of LMA (cf. Vitousek et al. 1990; Hultine and Marshall 2000; Kogami et al. 2001; Li et al. 2006), and factors affecting LMA are important for the variations of leaf δ^{13}C.

Altitudinal changes in the three leaf traits (leaf life span, LMA and N_{mass}) were different between the two deciduous broad-leaved species (*B. ermanii* and *S. commixta*) and the two evergreen *Abies* species. The two evergreen *Abies* species had longer-lived leaves at higher altitudes. The increase of leaf life span is adaptive to high altitudes for evergreen species by prolonging the time that individual leaves can actively assimilate CO_2 (Kikuzawa and Kudo 1995; Kudo 1999). Evergreen species tend to increase the leaf life span by increasing the LMA (i.e., mechanical strength). Leaf carbon is used for leaf structure, while leaf nitrogen is used for photosynthetic enzymes. Thus, the increase of LMA is related to a decrease in N_{mass}. As expected, it is considered that *A. mariesii* can maintain a positive carbon balance at high altitudes by compensating the reduction of N_{mass} (as a proxy of assimilation capacity) with the increase of the needle life span. By contrast, it is unclear why N_{mass} of *A. veitchii* increased with altitude, against the expectation that N_{mass} decreases with altitude. One of the possible reasons of the unexpected increase of N_{mass} is a measurement error. However, the increase of the leaf life span of *A. veitchii* with altitude is, at least, accordance with the theory that evergreen species adapt to high altitude by increasing leaf life span.

The relationship between LMA and leaf δ^{13}C was weaker in the two evergreen conifer species than the two deciduous broad-leaved species. Warren et al. (2003) also reported an unclear relationship between leaf δ^{13}C and LMA in a conifer Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) because LMA did not correlate with the transfer conductance that describes the internal conductance from intercellular spaces to the sites of carboxylation. Although these are few studies that examined transfer conductance of conifers (e.g., Warren et al. 2003, 2004), it is possible that the effect of LMA on the transfer conductance is not evident in conifers.

In general, leaf life span of deciduous trees decreases with increasing altitude (or decreasing growth period), but deciduous tree species can maintain a positive carbon balance at high altitudes by having leaves with low LMA (i.e., low construction cost per leaf area) and high N_{mass} (Kudo 1999; Taguchi and Wada 2001). The results of this study suggest that the two deciduous broad-leaved species adapted to high altitudes, as expected, except for the highest altitude. The reason of the unexpected increase of LMA of the two broad-leaved species at the highest altitude is unclear. Some researchers reported that strong wind and low temperature increase LMA to enhance the mechanical stiffness (cf. Coley 1983; Wardlaw et al. 1983; Woodward 1983; Körner et al. 1989). In general, wind velocity increases with increasing altitude (Araki 1995), associated with the decline in air temperature. Considering the increase of LMA of the two broad-leaved species at the highest altitude, LMA may increase to enhance mechanical stiffness if such a factor exceeds a threshold that the species can tolerate. Morecroft and Woodward (1990) suggested that altitudinal change of δ^{13}C is affected not only temperature and air pressure but also by the other environmental factors. Thus, it is possible that low temperature and strong wind affected the LMA of the two deciduous broad-leaved species at their altitudinal limit. In addition, the change of LMA must be accompanied with changes of leaf structure such as leaf thickness, spongy tissue, palisade mesophyll and epidermal tissue. Especially, internal conductance that influences foliar δ^{13}C is largely affected by surface area of chloroplasts exposed to intercellular airspace per unit leaf area (see Evans and Loreto 2000; Terashima et al. 2006 for review). Different changes of N_{mass} at the highest altitude between *B. ermanii* and *S. commixta* are probably ascribed to differences in anatomical changes. Thus, further anatomical studies are necessary to understand how LMA of two deciduous broad-leaved species increases at the highest altitude.

Overall, the different altitudinal patterns in the leaf traits between the two deciduous broad-leaved species and the two evergreen *Abies* species were ascribed to the difference in leaf longevity (the deciduousness and evergreenness), which in turn explains the different patterns in altitudinal changes of leaf $\delta^{13}C$ through LMA. Therefore, it is suggested that a functional relationship among leaf life span, LMA and assimilation capacity caused the different altitudinal patterns of leaf $\delta^{13}C$ between the deciduous and evergreen species.

Although the $\delta^{13}C$ of the two deciduous broad-leaved species decreased with altitude except for the highest altitude in this study, several studies showed that leaf $\delta^{13}C$ increases with altitude, irrespective of plant life form such as tree, shrub and herb species (Körner et al. 1988, 1991; Marshall and Zhang 1994). However, Körner et al. (1988, 1991) and Marshall and Zhang (1994) analyzed pooled data of many species of each life form along altitudinal gradients, and therefore, their results do not indicate that $\delta^{13}C$ of all individual species increases with increasing altitude. In fact, there is interspecific variation in altitudinal change in foliar $\delta^{13}C$. For example, foliar $\delta^{13}C$ decreased with altitude in *Pinus roxburghii* in Nepal (Sah and Brumme 2003), while the reverse pattern was observed in Hawaiian *Metrosideros polymorpha* (Vitousek et al. 1990). Körner (1999) ascribed the increase of foliar $\delta^{13}C$ with altitude to increased CO_2 drawdown in the leaf, related to the increase of N_{mass} and leaf thickness at high altitudes. Although the increases in both N_{mass} and leaf thickness with altitude in Körner's study do not correspond with the results of this study, the data of this study agrees with Körner's study in part, i.e. leaf $\delta^{13}C$ was positively correlated with leaf thickness and LMA. The results of this study therefore highlight the importance of leaf morphology as a control of $\delta^{13}C$ of leaves by lengthening the internal diffusion pathway to chloroplasts (Sprugel et al. 1996; Shipley et al. 2005).

This study demonstrates that the causal factor for the altitudinal variation in $\delta^{13}C$ was LMA in the examined four species. Moreover, the different altitudinal changes in leaf $\delta^{13}C$ between the two deciduous species (*B. ermanii* and *S. commixta*) and the two evergreen species (*A. veitchii* and *A. mariesii*) can be explained by the functional relationship among LMA, N_{mass} and leaf life span. Thus, our results show a significant approach for understanding altitudinal variations in foliar $\delta^{13}C$. However, our comparison did not exclude the effects of phelogeny on the leaf $\delta^{13}C$ due to the small number of species examined. Kelly and Woodward (1995) recommended taking taxonomic relatedness into account for comparison of $\delta^{13}C$. Thus, as a generalization, many species including both deciduous and evergreen species should be investigated to clarify the effects of taxonomic relatedness on interspecific variations in leaf $\delta^{13}C$ through functional relationships among LMA, N_{mass} and leaf life span.

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Figure legends

Fig. 1. Altitudinal distribution ranges (thick horizontal line) of *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on the east slope of Mount Norikura, central Japan. Open circles on the lines represent the altitudes examined in this study.

Fig. 2. Altitudinal changes of stable carbon isotope ratios ($\delta^{13}C$) of leaves of *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Different letters in each panel indicate significant differences ($P < 0.05$) by Tukey HSD test. Mean values ± SD are shown in each panel. Total numbers of samples were 25, 40, 15 and 15 for *B. ermanii*, *S. commixta*, *A. veitchii* and *A. mariesii*, respectively.

Fig. 3. Altitudinal changes of leaf mass per area (LMA) of *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Different letters in each panel indicate significant differences ($P < 0.05$) by Tukey HSD test. Mean values ± SD are shown in each panel. Total numbers of samples were 25, 40, 15 and 15 for *B. ermanii*, *S. commixta*, *A. veitchii* and *A. mariesii*, respectively.

Fig. 4. Relationship between stable carbon isotope ratio (δ^{13}C) and leaf mass per area (LMA) for *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Pearson correlation coefficient R is shown in each panel. Symbols are as follows, ○: 1600 m a.s.l., □: 1800 m a.s.l., ●: 2000 m a.s.l., ■: 2200 m a.s.l., ×: 2400 m a.s.l.

Fig. 5. Altitudinal changes of leaf nitrogen content per leaf mass (N_{mass}) of *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Different letters in each panel indicate significant differences ($P < 0.05$) by Tukey HSD test. Mean values ± SD are shown in each panel. Total numbers of samples were 25, 40, 15 and 15 for *B. ermanii*, *S. commixta*, *A. veitchii* and *A. mariesii*, respectively.

Fig. 6. Relationship between leaf nitrogen content (N_{mass}) and leaf mass per area (LMA) for *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Pearson correlation coefficient R is shown in each panel. Symbols are as follows, ○: 1600 m a.s.l., □: 1800 m a.s.l., ●: 2000 m a.s.l., ■: 2200 m a.s.l., ×: 2400 m a.s.l.

Fig. 7. Altitudinal changes of leaf life span for *Betula ermanii*, *Sorbus commixta*, *Abies veitchii* and *Abies mariesii* on Mount Norikura, central Japan. Different letters in each panel indicate significant differences ($P < 0.05$) by Tukey HSD test. Mean values ± SD are shown in each panel. Total numbers of samples were 150, 102, 45 and 45 for *B. ermanii*, *S. commixta*, *A. veitchii* and *A. mariesii*, respectively.

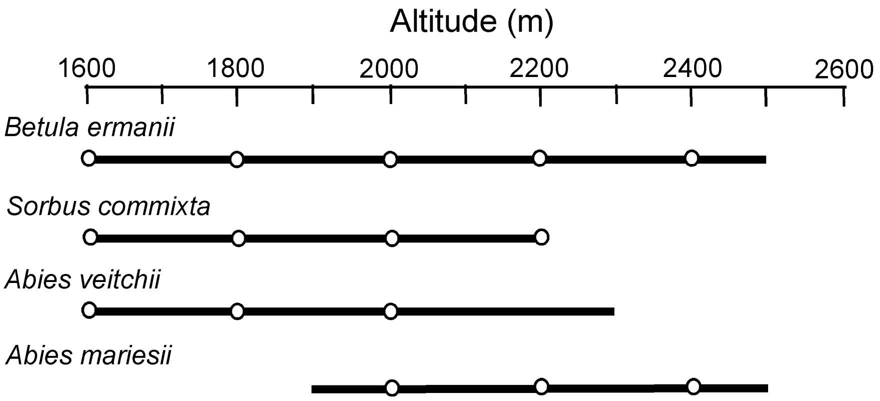
Altitude (m)
1600
1800
2000
2200
2400
2600
Betula ermanii
Sorbus commixta
Abies veitchii
Abies mariesii

Fig. 1

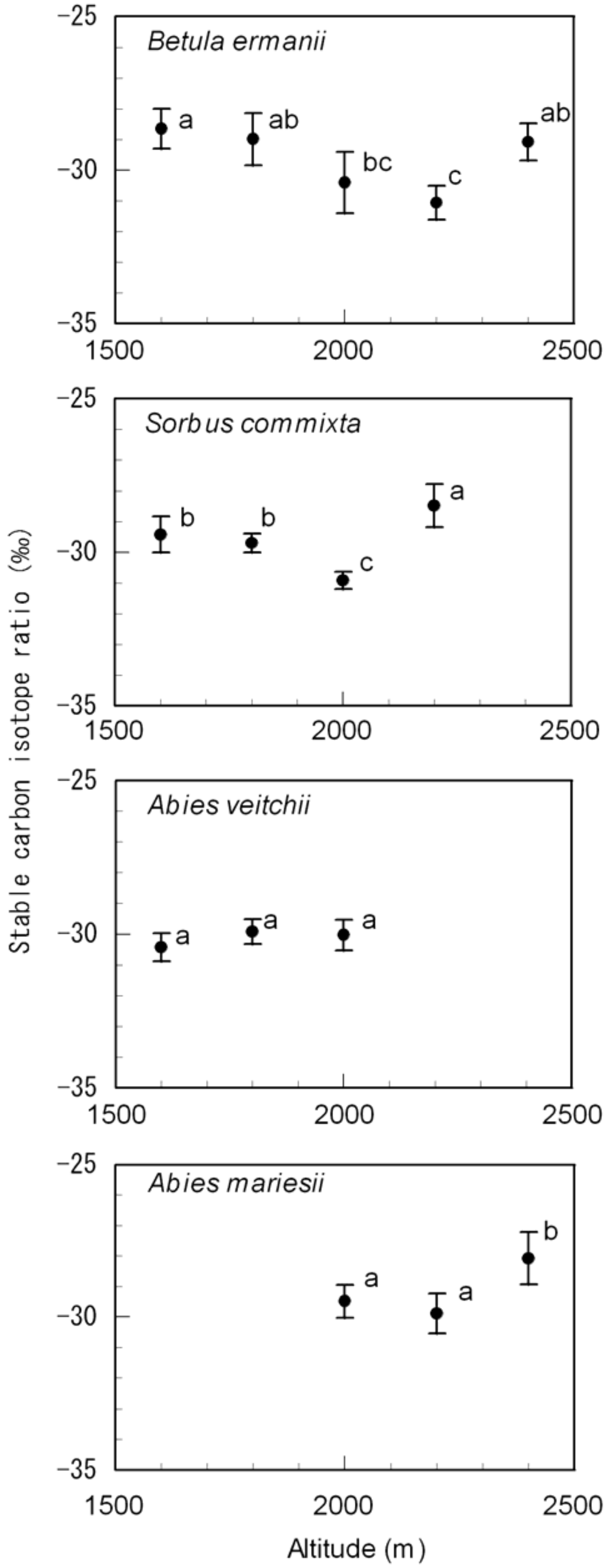
Stable carbon isotope ratio (‰)
Betula ermanii
a
ab
bc
c
ab
Sorbus commixta
b
b
c
a
Abies veitchii
a
a
a
Abies mariesii
a
a
b
−25
−30
−35
1500
2000
2500
Altitude (m)

Fig. 2

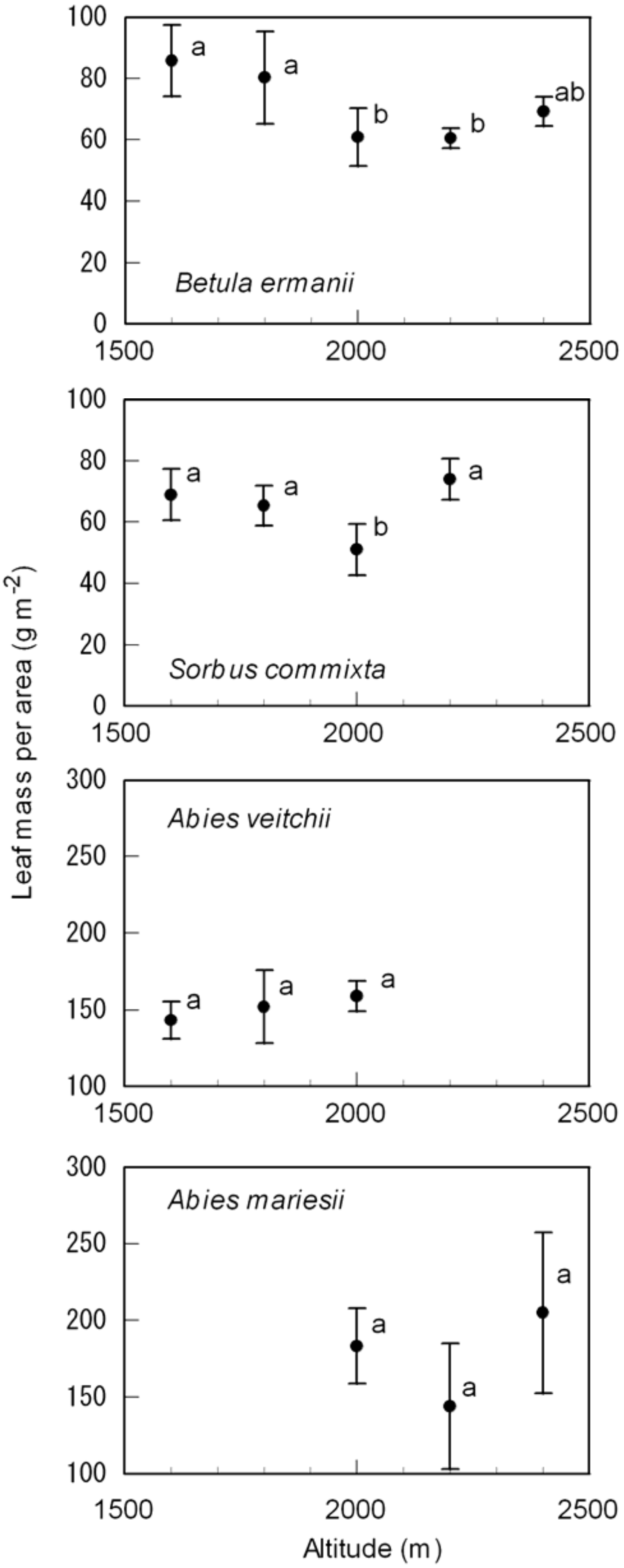
Leaf mass per area (g m^{-2})
Betula ermanii
a
a
b
b
ab
100
80
60
40
20
0
1500
2000
2500
Sorbus commixta
a
a
b
a
100
80
60
40
20
0
1500
2000
2500
Abies veitchii
a
a
a
300
250
200
150
100
1500
2000
2500
Abies mariesii
a
a
a
300
250
200
150
100
1500
2000
2500
Altitude (m)

Fig. 3

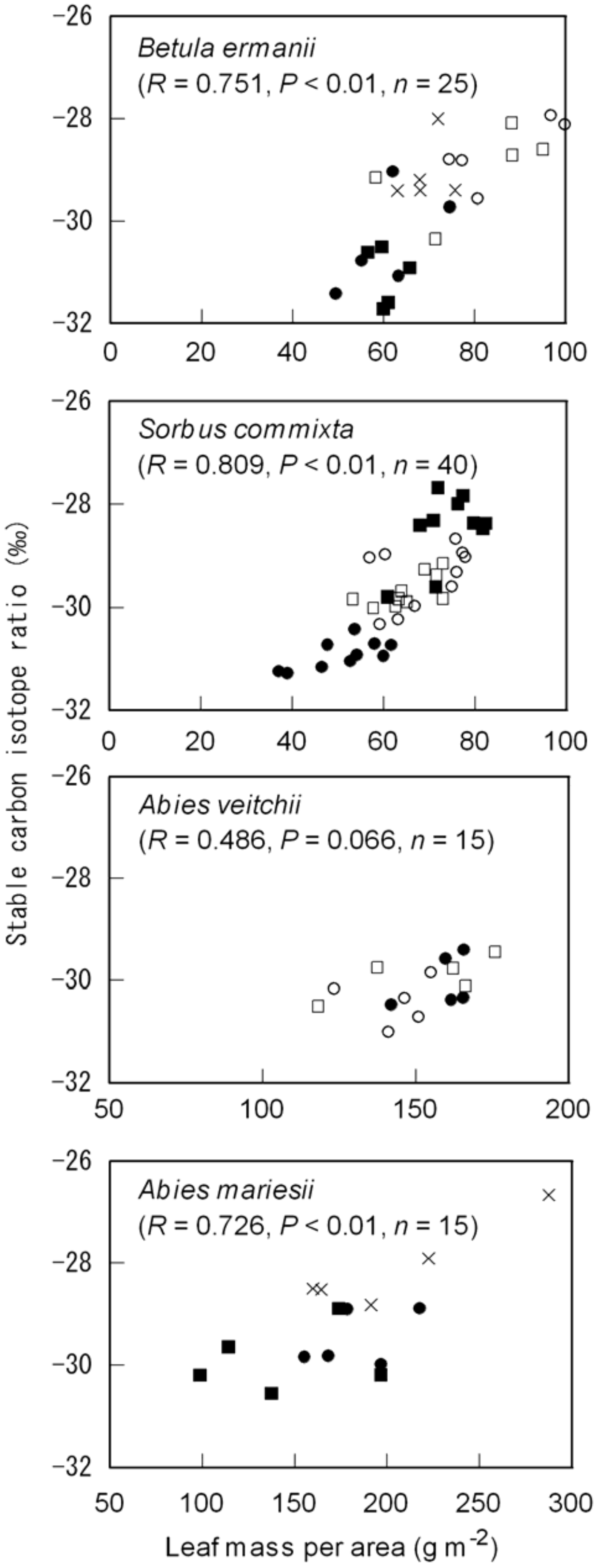
Betula ermanii
(R = 0.751, P < 0.01, n = 25)
Sorbus commixta
(R = 0.809, P < 0.01, n = 40)
Abies veitchii
(R = 0.486, P = 0.066, n = 15)
Abies mariesii
(R = 0.726, P < 0.01, n = 15)
Stable carbon isotope ratio (‰)
Leaf mass per area (g m^{-2})
−26
−28
−30
−32
0
20
40
60
80
100
50
100
150
200
250
300

Fig. 4

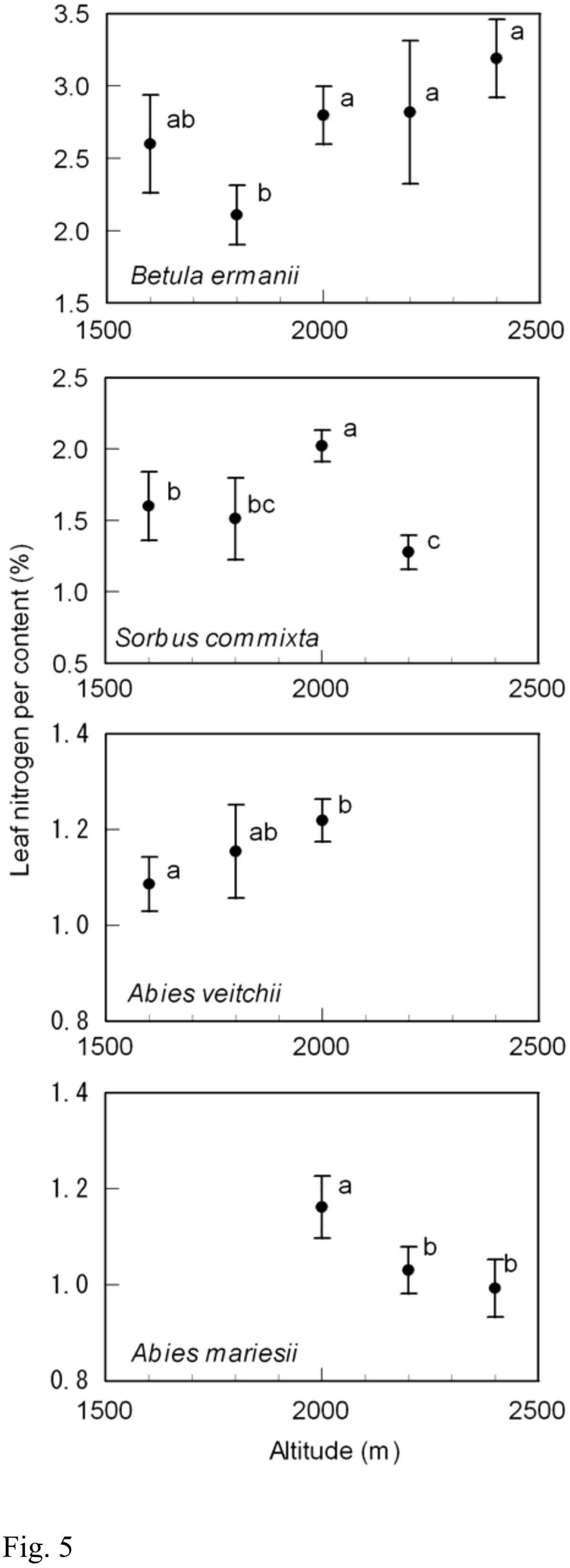
3.5
3.0
2.5
2.0
1.5
ab
b
a
a
a
Betula ermanii
1500
2000
2500
2.5
2.0
1.5
1.0
0.5
b
bc
a
c
Sorbus commixta
1500
2000
2500
1.4
1.2
1.0
0.8
a
ab
b
Abies veitchii
1500
2000
2500
1.4
1.2
1.0
0.8
a
b
b
Abies mariesii
1500
2000
2500
Leaf nitrogen per content (%)
Altitude (m)

Fig. 5

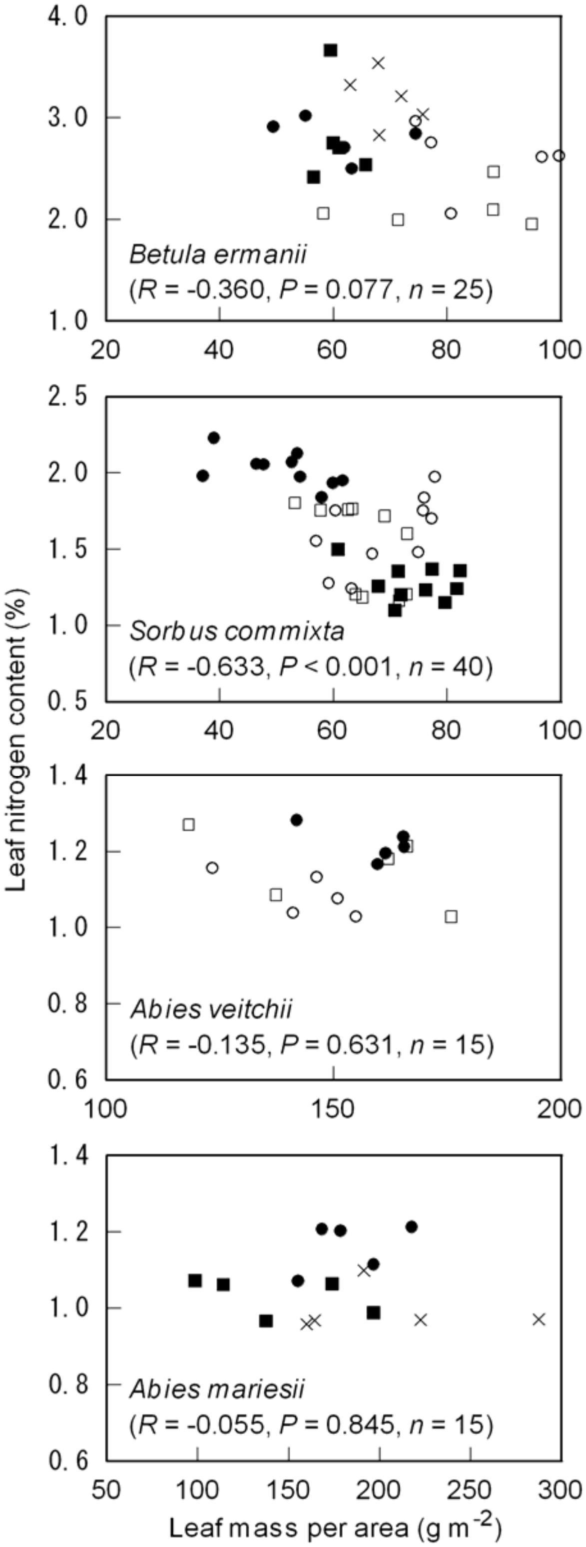
Leaf nitrogen content (%)
Leaf mass per area (g m^{-2})
Betula ermanii
(R = -0.360, P = 0.077, n = 25)
4.0
3.0
2.0
1.0
20
40
60
80
100
2.5
2.0
1.5
1.0
0.5
Sorbus commixta
(R = -0.633, P < 0.001, n = 40)
1.4
1.2
1.0
0.8
0.6
100
150
200
Abies veitchii
(R = -0.135, P = 0.631, n = 15)
50
250
300
Abies mariesii
(R = -0.055, P = 0.845, n = 15)

Fig. 6

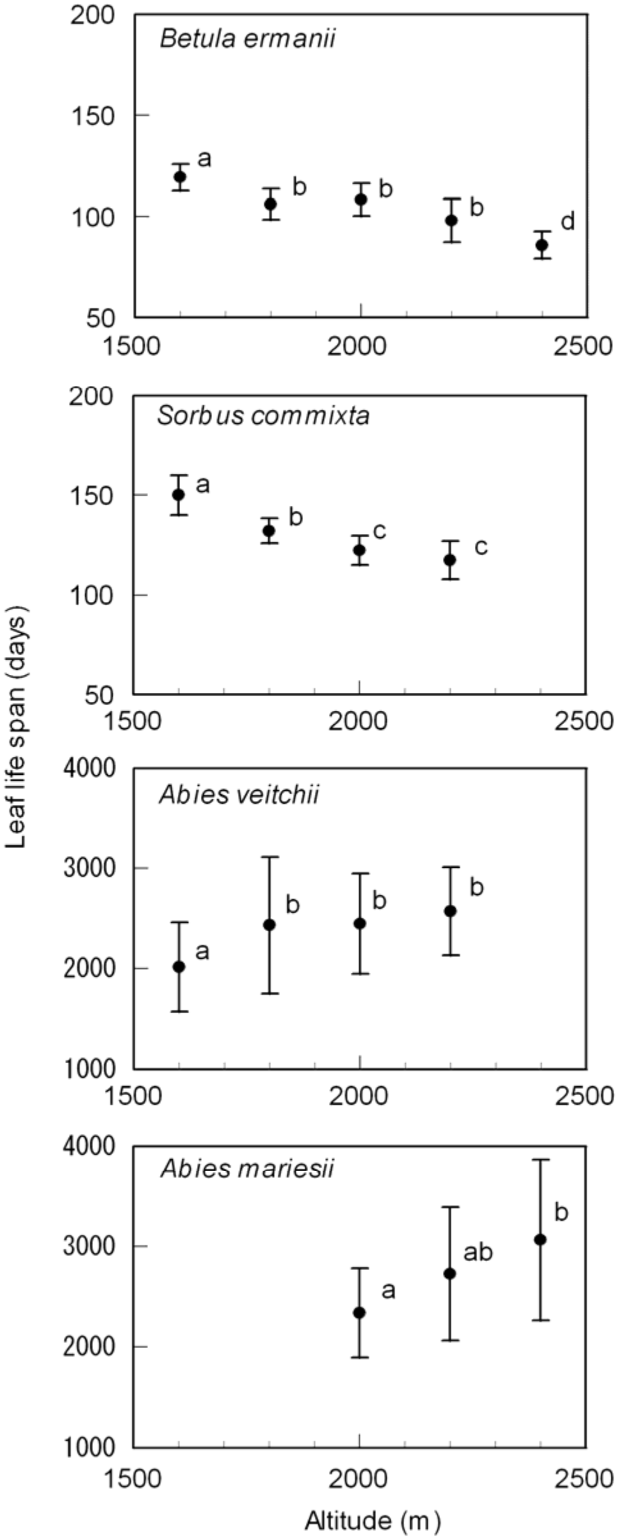
Betula ermanii
Sorbus commixta
Abies veitchii
Abies mariesii
Leaf life span (days)
Altitude (m)
200
150
100
50
4000
3000
2000
1000
1500
2000
2500
a
b
c
d
ab

Fig. 7