Plant Ecol (2022) 223:99–112
https://doi.org/10.1007/s11258-021-01192-8

One large tree crown can be defined as a local hotspot for plant species diversity in a forest ecosystem: a case study in temperate old-growth forest

Wakana A. Azuma · **Natsuki Komada** · **Yuya Ogawa** · **Hiroaki Ishii** ·
Akira Nakanishi · **Yuiko Noguchi** · **Mamoru Kanzaki**

Received: 21 January 2021 / Accepted: 27 September 2021 / Published online: 15 October 2021

Abstract Large, trees have survived for a long time, and their complex crown structure can serve as habitat for epiphytic plants. Canopy plants are not as well studied in the temperate zones as in the tropics, because many of them are accidental epiphytes, epiphytic individuals of normally terrestrial species. We hypothesized that the canopy can serve as a refuge for terrestrial species that have difficulty establishing on the ground (e.g., insufficient light, deer over-browsing), promoting and conserving forest species diversity. Terrestrial species may also vary in their ability to adapt to a wide range of canopy growth conditions. Here, we investigated canopy vascular plants hosted on a large *Cercidiphyllum japonicum* tree in a temperate old-growth forest, Japan. The canopy plant community was diverse comprising 39 vascular plant species (6 of which were threatened species), including 31 accidental epiphytes and 8 obligate epiphytes. We found at least 14 species could escape deer over-browsing by establishing in the canopy. Most accidental epiphytes were found on multiple and large reiterated trunks bifurcated from the main trunk as well as on large horizontal branches. Leaves of canopy plants had higher nitrogen concentration compared to those of plants on the ground, and higher water-use efficiency with increasing height was achieved by controlling transpirational water loss. Our results show one large tree crown can be defined as a local hotspot for current and future plant species diversity in a temperate old-growth forest, reinforcing its ecological value for conservation purposes.

Keywords Canopy plants · Epiphytes · Deer foraging · Ecophysiology · Conservation

Introduction

Large, old trees in old-growth forests, among the biggest and most long-lived organisms on earth, are important for their rarity and limited spatial distribution in the current global environment (Lindenmayer et al. 2012). Furthermore, branches and trunks of large old trees create complex canopy structure that provides physical habitat for other organisms (Sillett and Van Pelt 2007). Canopy plants, including epiphytes are found in various climatic zones. For example, in

Communicated by Lauchlan Fraser.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11258-021-01192-8.

W. A. Azuma (✉) · H. Ishii · Y. Noguchi
Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo 657-8501, Japan
e-mail: wakana@port.kobe-u.ac.jp

W. A. Azuma · N. Komada · Y. Ogawa ·
A. Nakanishi · M. Kanzaki
Graduate School of Agriculture, Kyoto University,
Kyoto 606-8502, Japan

tropical montane or lowland forests, vascular epiphytes are dominant and important for species richness, as well as for their role in water and nutrient cycling of forest ecosystems (e.g., Krömer et al. 2005; Hsu and Wolf 2009). In temperate forests of the Pacific Northwest Coast of North America, high diversity of epiphytes especially ferns, bryophytes, and lichens were documented in the crowns of large trees in old-growth conifer forests (e.g., McCune et al. 1997; Williams and Sillett 2007; Gorman et al. 2019). In boreal forests, epiphytic lichens on large, old trees greatly contribute to increasing local biodiversity and nutrient cycling, facilitating establishment of other organisms (e.g., Essen et al. 1996; Lie et al. 2009).

In temperate forests, however, canopy plants are not as well studied as they are in the tropics. This is because, while research on canopy plants generally focuses on obligate epiphytes (holoepiphytes) that thrive only on trees, they are less common in the temperate zone (Zotz 2005). Most anecdotally and frequently reported in the temperate zone are accidental epiphytes, which are epiphytic individuals of terrestrial plant species (Benzing 2004; Hoeber et al. 2019). Because moisture is an important factor facilitating establishment of accidental epiphytes, they are often found in wet forests growing on large, old trees with accumulations of organic material (arboreal soil, sensu Nadkarni et al. 2002). However, water availability alone does not seem to explain abundance and composition of accidental epiphyte communities (Hoeber et al. 2020). Development of crown structure of the host tree is another factor contributing to canopy plant diversity. The trunks and branches that grow thicker with tree age contribute to increasing surface area for establishment of canopy plants and the accumulation of arboreal soils, a regulating factor for size and abundance of canopy plants (Sillett and Van Pelt 2007). Therefore, the long-term development of structures in the host tree would be one of the key factors in determining the canopy plant diversity.

The canopy could also function as a refuge for some terrestrial plants that may have difficulty establishing on the ground. For example, in an old-growth warm-temperate forest in southern Japan, the canopy environment is important for maintaining species diversity by providing growing conditions not available on the forest floor, such as high light intensity necessary for survival of shade-intolerant species (Ishii et al. 2018). The impact of excessive deer foraging of forest floor vegetation is a serious issue in various forest ecosystems (e.g., Côté et al. 2004; Beguin et al. 2016) including Japan (Takatsuki 2009). Thus, if deer-preferred terrestrial species become accidental epiphytes, the canopy could potentially contribute to persistence of species whose growth and reproduction on the forest floor are limited by over-browsing.

For being able to survive in the canopy, some terrestrial species may have acquired ecophysiological characteristics that allow them to adapt to a wide range of growing conditions. Temperature and light intensity generally increase and air humidity decreases from the forest floor toward the canopy and microclimatic conditions are more variable in the canopy than on the forest floor (Parker 1995; Shaw 2004). Water availability in particular can be a major limiting factor for growth and survival of canopy plants (Laube and Zotz 2003; Hoeber et al. 2019). To maintain physiological function, canopy plants must be able to tolerate or acclimate to these growing conditions.

Here, we investigated species composition and ecophysiological characteristics of canopy vascular plants hosted in the crown of a large *Cercidiphyllum japonicum* Siebold & Zucc. tree in a temperate old-growth forest, Japan. This tree shows a prominently developed tree architecture with abundant arboreal soil, which has been shown to have similar nitrogen availability as terrestrial soil (Tatsumi et al. 2021). To examine how canopy plant diversity is related to host tree structure and environmental conditions, we first documented the three-dimensional architecture of the host tree and vertical temperature gradient in the crown. Second, we identified all canopy vascular plants on the host tree using rope climbing techniques that are highly reliable for direct observation of epiphytes (Miller et al. 2017). Third, we compared ecophysiological characteristics between plants growing in the canopy and on the ground to understand how they adapt to different conditions. To this end, water and nutrient use efficiencies were estimated by measuring leaf functional traits such as carbon (C) and nitrogen (N) concentrations and stable isotope compositions of leaves collected from various heights in the crown of the host tree. Stable carbon isotope composition of leaves is a good indicator of photosynthetic water-use efficiency (Farquhar et al. 1989). When plants are under water-deficient conditions, closing of stomata to prevent transpirational water loss comes at the cost of reduced CO_2 uptake for photosynthesis, resulting in higher values of δ^{13}C (Monclus et al. 2006). High water-use efficiency can also be achieved by high photosynthetic capacity associated with higher leaf N concentrations (Hikosaka et al. 1998; Takashima et al. 2004). We hypothesized that canopy plants, whose distribution is limited by water availability, would show higher water-use efficiency than plants growing on the forest floor. The δ^{15}N of plant tissue reflects isotope fractionation during N uptake and N assimilation, as well as δ^{15}N of the N sources (Handley and Raven 1992; Evans 2001), allowing us to infer N-use efficiency as well as the source of N for epiphytes on the host tree. Our final objective was to evaluate the ecological value of the host tree and to understand how canopy plants adapt to epiphytic habitats for purposes of conservation of vascular plant diversity in the temperate, old-growth forest.

Materials and methods

Study site and host tree

The study was conducted in the Ashiu Research Forest of Kyoto University, which is one of the finest natural forests in western Japan and part of the Kyoto Tamba Kogen Quasi-National Park (35°18′N, 135°43′E; 355–959 m a.s.l.). This forest is located in the transition zone between cool-temperate and warm-temperate forests, and more than half of the forest area is old-growth. There is heavy snowfall ranging from 1 to 3 m in winter. The mean monthly temperature ranges from − 1.1 °C in January to 22.5 °C in August, the mean monthly precipitation ranged from 129 mm in February to 309 mm in September, and the mean annual precipitation is 2280 mm at the forest weather station at 640 m a.s.l. from 2000 to 2015.

Our study tree (host tree) was a *C. japonicum* (38 m tall and ca. 3.5 m in diameter at breast height) which is one of the largest trees in the forest reserve. The oldest record of the host tree is a photograph taken in 1928 showing that it had its current shape at that time (Field Science Education and Research Center of Kyoto University, personal communication). Hence, the age of the host tree is estimated to be over 100 years old. There are 17 large and distinctively shaped trees (consisting of 12 species) designated as preserved trees in the Ashiu Research Forest, 2 of which are *C. japonicum* including the host tree (Ashiu Research Forest Research Group 1987). The host tree is located at 640 m a.s.l. in a cool temperate, riparian, old-growth forest. In such forest areas in Ashiu, it is dominated by *Aesculus turbinata* Blume and *Pterocarya rhoifolia* iebold & Zucc., while *Cryptomeria japonica* (L.f.) D.Don f. *radicans* (Nakai) Sugim. et Muroi and *Fagus crenata* Blume dominate the upper and lower slopes, respectively (Yamanaka et al. 1993). *C. japonicum* usually clusters with *A. turbinata* and *P. rhoifolia*, and large individuals (DBH > 1.0 m), having multiple stems and large crowns like the host tree, are one of the dominating characteristics of the riparian zone. In recent years, over-browsing by deer has had serious negative impact on recruitment and diversity of understory vegetation, as well as on the animal and insect fauna that utilize it (Kato and Okuyama 2004; Fukuda and Takayanagi 2008; Sakaguchi et al. 2008; Fujii 2010).

Assessment of tree architecture and vertical temperature variation in the host tree

We accessed the crown of the host tree non-destructively using single and double-rope climbing techniques. To reconstruct the three-dimensional structure of the host tree, the tree architecture was mapped in three-dimensional coordinate space using a hand-held laser range finder (TruePalse360, Laser Technology, Inc., USA) according to the methods described in Nakanishi et al. (2013, 2016). We measured from one of five reference points: two on the ground (ca. 10 m from the trunk base) and three on the host tree. Measured points were as follows: the base of the trunk, nodes, and ends of branches (see Kramer et al. (2018) for definition of measured elements). Nodes of small branches less than about 10 cm in diameter were omitted because it is difficult to accurately measure their dimensions. Slope distance, vertical angle, and horizontal angle from the reference point to the measured point were recorded. The sections between two measured points were defined as segments and the tree was considered as an aggregate of these segments.

In summer of 2017 (total of 58 days during June to August), vertical temperature changes within the crown were measured at hourly intervals using thermal sensors (Thermochron Type-G, KN Laboratories, Inc., Japan) which were installed in the crown of the host tree at 0, 5, 10, 15, 20, 26, 30, and 33 m above ground. One temperature sensor was installed at each height so that they were all at the similar distances from the tree trunk. To avoid the effects of direct sunlight, we covered the sensor with aluminum foil.

Canopy plant inventory

Canopy plants were inventoried from May to November 2017. A tape measure was stretched vertically from treetop to ground to determine the height of each plant from the ground. All vascular plants observed in the crown of the host tree were then recorded as canopy plants with their species name and the height of their stem base. Lianas germinate on the ground and later climb up trees to expand their plant body to the canopy, making it difficult to determine whether they are canopy or terrestrial plants (Zotz 2013a). Here, we categorized them as canopy plants following the criteria of Nadkarni et al. (2001). It was difficult to identify the rooting point and distinguish individual plants for lianas, which are connected from the forest floor along the trunk of the host tree. The lianas found in the upper canopy on the host tree often rooted in arboreal soils. Based on these observations, the height of lianas in this study were recorded where the leaves occurred. It was also difficult to distinguish individual plants for ferns and herbs because some of them formed dense patches comprising multiple individuals. Trees and shrubs formed sparse patches consisting of one to four individuals.

Canopy plant species were categorized according to life-form (trees, shrubs, lianas, and herbs), threatened/non-threated species, accidental/obligate epiphytes, deer preferred/not preferred, and the heights of their location on the host tree was measured. Threatened species were defined according to the Red Data Book of Kyoto Prefecture (Kyoto Prefecture 2015) and the Japanese Red Lists on 9 Taxonomic Groups (4th edition, Ministry of the Environment Government of Japan 2019). The Japanese Red List categories and the IUCN's categories are basically the same. However, the Japanese list does not have a category of "least concern (LC)", while it contains an original category, "local population (LP)". Deer-preferred species were defined with reference to reports by Fukuda and Takayanagi (2008) and Sakaguchi et al. (2012a, b), which investigated the effect of deer browsing in the Ashiu Forest Reserve.

Leaf ecophysiological characteristics

To compare leaf ecophysiological characteristics between plants growing in the canopy and on the forest floor (hereafter: canopy and ground plants, respectively), we sampled 43 leaves from 16 species of canopy plants and 17 leaves from 7 species of ground plants (including 5 leaves of the host tree sampled from various heights) in August 2017 and June 2018. The sampled leaves represented 10 tree species (excluding the host tree), 3 shrub species, 3 herbaceous species (including 2 ferns), and 4 liana species (Online Resource 1). Sampling reflected the abundance of species in each category on the host tree: *Davalia mariesii* accounted for 13 of 17 leaves of herbaceous species; *Hydrangea petiolaris* and *Hydrangea hydrangeoides* accounted for 11 and 6, respectively, of 18 leaves of lianas.

After collection, the sample leaves were dried to constant weight at 60 °C for more than 48 h, ground to fine powder, and loaded into capsules for isotope analysis. Measurements of total N and C concentrations and stable isotope ratios were made using an isotope ratio mass spectrometer (Delta V IRMS, Thermo Fisher Scientific, USA) connected to an elemental analyzer. The precision of the on-line procedure was better than ± 0.2‰ for the isotope ratio. Natural abundance of ^{15}N and ^{13}C was expressed in per mil (‰) deviation from international standards: δ^{15}N or δ^{13}C = $[R_{sample}/R_{standard} - 1] \times 1000$, where R is ^{15}N/^{14}N or ^{13}C/^{12}C, respectively. Atmospheric N and Pee Dee Belemnite (PDB) were used as the standards for N and C, respectively.

Data analysis

Three-dimensional architecture of the host tree was depicted using R Package 'plot 3D' (Soetaert 2017) with R software v. 3.4.0 (R Development Core Team 2017). A minimum, mean, and maximum temperature and mean daily temperature difference were calculated for each temperature sensor. Vertical trends in temperature were tested using one-way analysis of variance (ANOVA) followed by Tukey's HSD test.

Leaf ecophysiological characteristics were analyzed in an analysis of covariance (ANCOVA) with leaf height or leaf δ^{13}C as the covariate and growing habitat (canopy/ground) and life-form (trees, shrubs, lianas, and herbs) as categorical independent variables. We used JMP14 (SAS Institute, Cary, NC, USA) for all analyses.

Results

Tree architecture and vertical temperature variation

The three-dimensionally reconstructed architecture of the host tree as viewed facing north and west is shown in Fig. 1. From the ground to a height of around 5 m, the tree had a single large trunk and the diameter at a height of 1.3 m was 350 cm and that at a height of 3.55 m was 279 cm. Above this point, several reiterated trunks arose between heights of around 5 m and 10 m, including four trunks reaching a height of over 20 m whose diameter at a height of 15 m was 33, 60, 73, and 77 cm. Between heights of around 10 m and 15 m, most of the reiterated trunks had no horizontal branches. Long horizontal branches appeared at above ca. 15 m, and then number of segments increased above ca. 20 m (Fig. 2).

The mean temperature and the mean daily temperature difference in summer increased with height (Fig. 3). The mean temperature ranged from 20.5 °C at a height of 0 m, to 21.5 °C at a height of 20, 26, 30, 33 m.

Canopy plant community

We found 39 species of vascular plants on the host tree, including 12 tree species, 12 shrub species, 4 liana species, and 11 herb species (including s7 ferns) (Online Resource 2). All tree, shrub, and liana species, except for *Ribes ambiguum* Maxim. and 7 herb species were accidental epiphytes. There were 6 threatened species, some of which were obligate epiphytes found only in the crown of large, old trees. At least 14 species affected by deer over-browsing on the forest floor were found in the crown of the host tree.

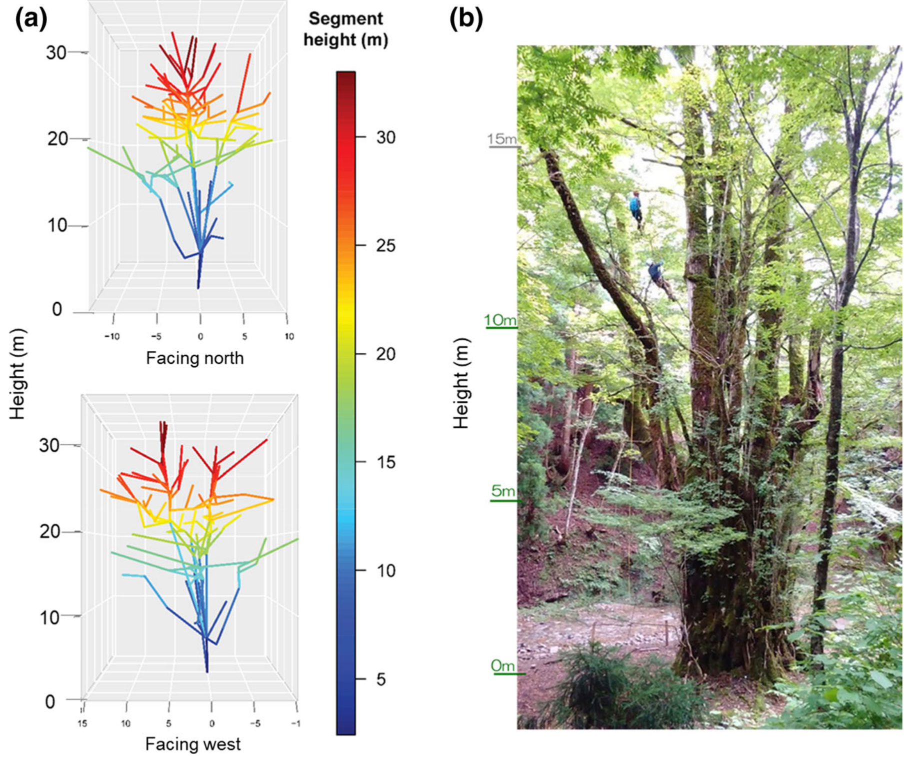

Fig. 1 **a** Three-dimensionally reconstructed architecture of the host tree as viewed facing north and west drawn using R Package 'plot 3D'. Color bars indicate the height of each segment. **b** The photo of the host tree facing south. Note climbers on rope in the photo for scale

The canopy plants showed wide vertical distribution on the host tree, ranging in height from the trunk base to 28 m (Online Resource 2). The greatest number of canopy plant species (18 species) occurred in the lower crown at heights ranging 5–10 m. Most of the tree and shrub species occurred in this height range. The greatest number of species of lianas and herbs occurred in the upper crown at heights ranging 20–25 m. Two liana species (Hydrangeaceae) and three fern species (Davalliaceae and Polypodiaceae) showed especially wide vertical distribution, with the difference between highest and lowest points observed being more than 15 m. All threatened species occurred in the upper crown at heights ranging 20–28 m.

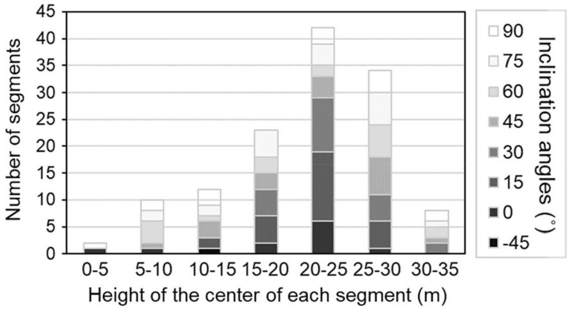

Fig. 2 Vertical distribution and inclination angle (relative to horizontal) of segments, which are the elements of the three-dimensionally reconstructed architecture of the host tree. Values indicate the number of segments. Heights and angles were measured at the center of each segment

Leaf ecophysiological characteristics of canopy plants

Leaf δ^{13}C increased with leaf height for both canopy plants and ground plants (Fig. 4a, Table 1). For the canopy plants, leaf N concentration (Fig. 4b) and leaf δ^{15}N (Fig. 4c) decreased, while leaf C:N ratio (Fig. 4d) increased with leaf height, whereas for the ground plants, these variables did not vary with leaf height (Table 1). Trends observed for leaf traits in relation to leaf δ^{13}C were opposite for canopy and ground plants as indicated by significant interaction terms for ANCOVA (Fig. 4, Online Resource 3). For example, leaf N concentration decreased with increasing leaf δ^{13}C for the canopy plants, whereas it increased for the ground plants (Fig. 4e, Table 1). There was no significant effect of growing habitat on leaf δ^{13}C and leaf δ^{15}N in relation to leaf height, while leaf N concentration was higher and leaf C:N ratio was lower for canopy plants compared to ground plants (Fig. 4, Online Resource 3). There was no effect of growing habitat on leaf δ^{15}N in relation to leaf δ^{13}C, while leaf N concentration was higher and leaf C:N ratio was lower in canopy plants compared to ground plants (Fig. 4,Online Resource 3).

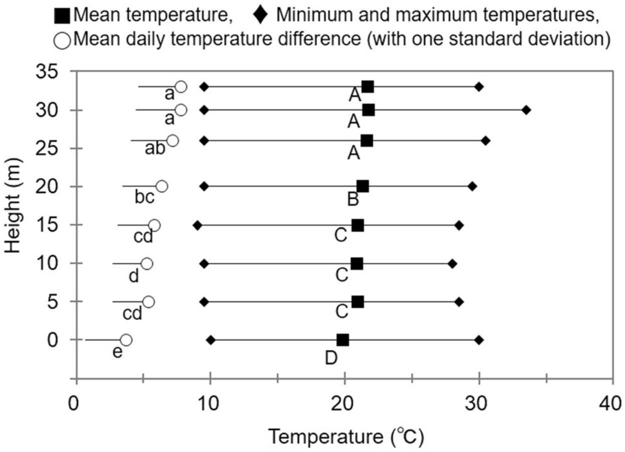

Fig. 3 Vertical variation of mean, minimum, maximum temperatures, and the mean daily temperature difference in relation to height in the host tree in summer 2017 (total of 58 days during June to August). Bars associated with daily mean temperature difference indicate one standard deviation. Different capital and small letters denote significant differences among the mean temperature and the mean daily temperature difference, respectively ($p < 0.001$, ANOVA followed by Tukey's HSD test)

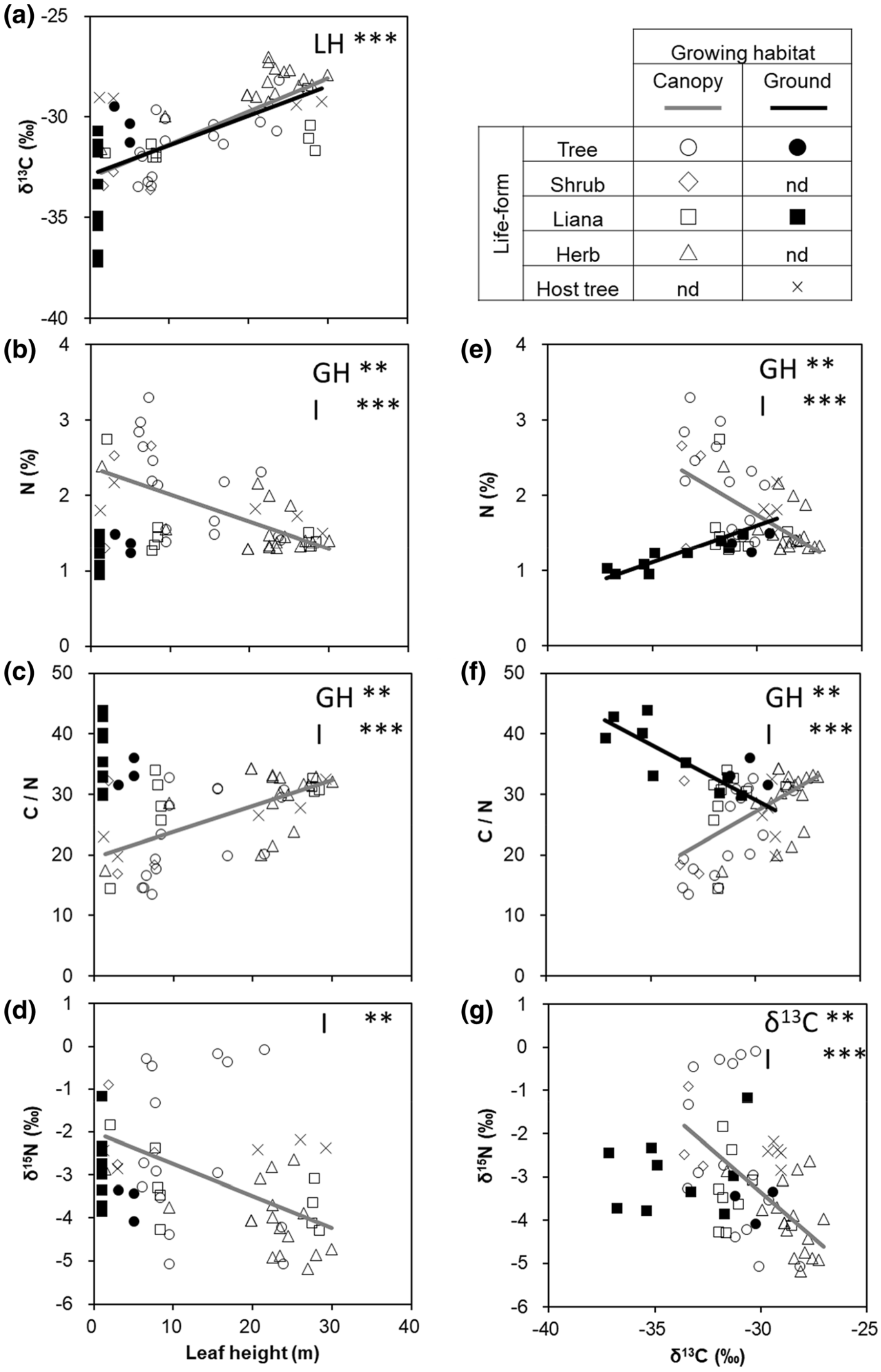

Fig. 4 The effect of growing habitat (canopy/ground) on leaf ecophysiological characteristics (compositional deviation of ^{13}C (δ^{13}C), nitrogen concentration (N), C:N ratio, and compositional deviation of ^{15}N (δ^{15}N) in relation to leaf height (**a**–**d**) and leaf δ^{13}C (**e**–**g**). Open and closed symbols indicate canopy plants and ground plants, respectively. Symbol shapes indicate plant life-forms and lines indicate significant linear regressions (see legend). The results of ANCOVAs with leaf height (LH) or leaf δ^{13}C (δ^{13}C) as covariate and growing habitat (GH) as the independent variable, and their interaction (I) are shown in each panel (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). See Table 1 and Online Resource 3 for the detailed results of ANCOVA

Table 1 Results of ANCOVA of leaf ecophysiological characteristics in relation to leaf height or leaf δ^{13}C (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Independent variable	δ^{13}C		N content				C:N ratio				δ^{15}N			
	Leaf height		Leaf height		δ^{13}C		Leaf height		δ^{13}C		Leaf height		δ^{13}C	
	R^2	P value	R^2	P value	R^2	P value	R^2	P value	R^2	P value	R^2	P value	R^2	P value
Growth habitat														
Canopy (n = 44)	0.57	***	0.31	***	0.30	***	0.31	***	0.31	***	0.20	***	0.31	***
Ground (n = 17)	0.19	*	0.10		0.67	***	0.02		0.63	***	0.03		0.04	
Plant life-form														
Tree (n = 18)	0.14		0.07		0.44	**	0.03		0.47	***	0.002		0.15	
Shrub (n = 3)	0.25		0.52		0.05		0.35		0.16		0.30		0.20	
Liana (n = 18)	0.31	**	0.004		0.17	*	0.04		0.35	**	0.16		0.01	
Herb (n = 17)	0.63	***	0.26	*	0.26	*	0.22	*	0.24	*	0.18	*	0.15	
Host tree (n = 5)	0.14		0.43		0.05		0.78	*	0.15		0.32		0.20	

Leaf ecophysiological characteristics also varied by life-form. Leaf δ^{13}C increased with leaf height for lianas and herbs (Fig. 5a, Table 1), while leaf N concentration and leaf δ^{15}N decreased with height for herbs (Fig. 5b, Table 1), and leaf C:N ratio increased for herbs and the host tree (Fig. 5c, Table 1). Trends observed for leaf traits in relation to leaf δ^{13}C were opposite for lianas compared to others life-forms as indicated by significant interaction terms for ANCOVA (Fig. 5, Online Resource 3). For example, leaf N concentration decreased with increasing leaf δ^{13}C for trees and herbs, whereas it increased for lianas (Fig. 5e, Table 1). Leaf C:N ratio increased with increasing leaf δ^{13}C for herbs and trees, whereas it decreased for lianas (Fig. 5f, Table 1). There was no significant effect of life-form on leaf δ^{15}N in relation to leaf height, while significant effects were observed for leaf δ^{13}C, leaf N concentration, and leaf C:N ratio (Fig. 5, Online Resource 3). There was no effect of life-form on leaf N concentration, leaf C:N ratio, and leaf δ^{15}N in relation to leaf δ^{13}C (Fig. 5, Online Resource 3).

Discussion

Canopy plant community

Our study demonstrated that a single large, old tree can contribute to plant species diversity in an old-growth temperate forest of East Asia. Compared to the previous reports in Japan, where a single large tree (DBH > 1 m) hosted 20 woody plant species in *C. japonica* (Ishii et al. 2018), 16 vascular plant species in *Quercus acuta* (Tochimoto et al. 2008), and 20 vascular plant species in *Quercus crispula* Blume (Kondo et al. 2008), the host tree in this study, *C. japonicum*, hosted 39 vascular plant species on the crown. In tropical forests, obligate epiphytes, including orchids and ferns, generally dominate canopy vascular plant communities (Kreft et al. 2004; Krömer et al. 2005; Zotz 2005; Hsu and Wolf 2009). Our study tree also hosted obligate epiphytes, most of which were threatened species. Dominant species hosted above a height of 20 m were epiphytic ferns, which are the most speciose group in temperate-zone epiphyte communities (Zotz 2005). Microhabitat heterogeneity within crowns increase as trees grow three-dimensionally, contributing to diversity and variability of epiphyte community structure (Sillett and Antoine 2004; Woods et al. 2015). Globally, large trees and old-growth forests are scarce, especially in temperate forests of heavily populated regions, which could seriously affect the long-term conservation of epiphyte communities (Kartzinel et al. 2013; Lindenmayer and Laurance 2017; Ortega-Solís et al. 2020). Conservation of large, old trees and their canopy habitats would substantially increase the plant diversity of local forest ecosystems.

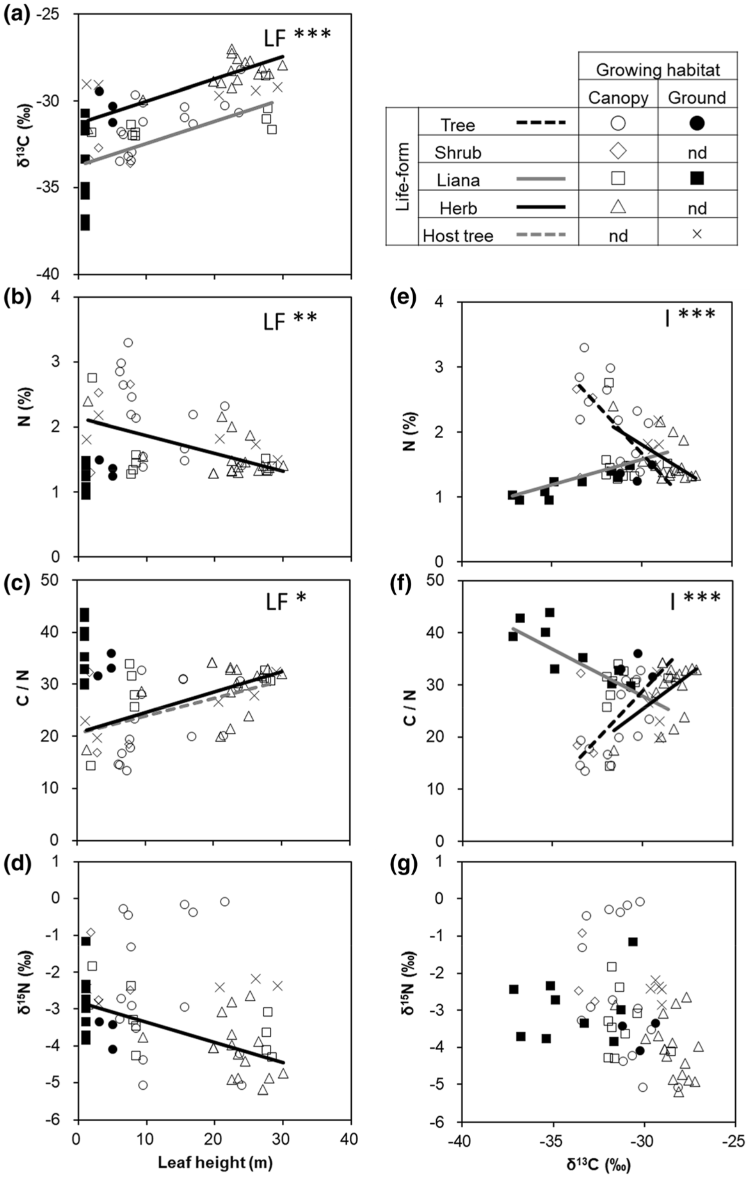

Fig. 5 The effect of life-form (tree, shrub, liana, herb, host tree) on leaf ecophysiological characteristics (compositional deviation of ^{13}C (δ^{13}C), nitrogen concentration (N), C:N ratio, and compositional deviation of ^{15}N (δ^{15}N) in relation to leaf height (**a**–**d**) and leaf δ^{13}C (**e**–**g**). Open and closed symbols indicate canopy plants and ground plants, respectively. Symbol shapes indicate plant life-forms and lines indicate significant linear regression (see legend). The results of ANCOVAs with leaf height (LH) or leaf δ^{13}C (δ^{13}C) as covariate and life-form (LF) as the independent variable, and their interaction (I) are shown in each panel ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). See Table 1 and Online Resource 3 for the detailed results of ANCOVA

On the other hand, nearly 80% of canopy vascular plant species hosted on our study tree (31 of 39 species) were accidental epiphytes. High occurrence of accidental epiphytes is also a common feature in the other temperate forests, such as in Europe, Chile, New Zealand, and the Himalayas (Zotz 2005; Hoeber et al. 2019). Among the few reports of canopy vascular plants in temperate forest of Japan, accidental epiphytes were rare and obligate epiphytes such as orchids and ferns were common in warm-temperate evergreen (lucidophyllous) natural forests in southern Japan (Hattori et al. 2007a, b, 2009; Tochimoto et al. 2008; Seto et al. 2020). In contrast, accidental epiphytes dominated in a cool-temperate forest in the central Japan dominated by *Fagus crenata* (Kondo et al. 2008), as well as in a cool temperate, mixed conifer-broadleaf forest dominated by *C. japonica* at 1050 m elevation in Yakushima Island, southern Japan (Ishii et al. 2018). These observations suggest that, in Japan, obligate epiphytes decrease in cooler climates. Similary, lower temperatures have been reported to be a major factor limiting the distribution of tropical (lowland) epiphyte taxa at higher altitudes (Hall 1958; Zotz 2005). Although the distribution of obligate epiphytes may be limited by low temperature, epiphytism of vascular plants per se is not necessarily limited by these low temperatures as illustrated by the abundance of accidental epiphytes in cool climates (Hoeber and Zotz 2021).

Host tree structures, such as large surface area, contribute to the occurrence of mat-like canopy plant communities (Nakanishi et al. 2013, 2016). In this study, high numbers of canopy plant species, including many accidental epiphyte tree species, were found in the lower crown at heights ranging 5–10 m where multiple and large reiterated trunks occurred, as well as on large horizontal branches of the host tree. These multiple reiterated trunks in large trees of *C. japonicum* is characteristic of this species, which extends its longevity by basal sprouting (Kubo and Sakio 2020). On these host tree structures, we found depositions of arboreal soils (ca. 10 cm deep), which have similar nitrogen availability to ground soils (Tatsumi et al. 2021), suggesting long-term accumulation of organic matter facilitates establishment of accidental epiphyte by providing nutrients and substrate for roots. In the upper crown, herbs were especially abundant at heights ranging 20–25 m where branches with an inclination of 0–45° were most numerous. On the other hand, lianas and ferns, such as Hydrangeaceae and Davalliaceae, established vertically along the large surface area of the trunk. Our findings reinforce the importance of age, surface area, and three-dimensional structure of the host tree in creating a rich substrate for canopy vascular plants.

Furthermore, our study suggested that crowns of large, old trees could function as a refuge for deer-preferred species to escape over-browsing. The pronounced negative impact of over-browsing on regeneration and establishment of deer-preferred plants on the forest floor has been shown using experimentally installed deer fences (Baines et al. 1994; Rooney et al. 2000; Sakaguchi et al. 2012a). Long-term over-browsing could affect soil seed banks limiting the potential for deer-preferred species to recover on the forest floor after deer density is controlled (Tamura 2016). Because of constraints on their growth, accidental epiphytes may not necessarily complete their life cycle epiphytically (Zotz 2013b). In the canopy of large *C. japonica* trees in Yakushima Island, Japan, however, several accidental-epiphyte species reproduced and maintained viable populations (Ishii et al. 2018). In our study tree, we observed that some woody species grew larger than 5 m in height, and according to the local ecotourism guides, several species were observed to flower and fruit every year in the canopy. If enough epiphytic individuals of a deer-preferred species grow to reproductive size in the canopy, they could potentially function as seed sources for future recolonization on the forest floor. Our results emphasize the importance of conserving multiple large, old trees for maintaining viable populations of endangered species, as well as those affected by over-browsing.

Canopy plant ecophysiology

Our analyses of carbon stable isotope composition of leaves suggested that leaf water-use efficiency increased with increasing height for both the ground and canopy plants. Ferns, which were mostly distributed in the upper-most part of the crown (20–30 m) showed particularly high leaf δ^{13}C values. Temperature was highest and daily temperature difference most variable in the upper-most crown. In addition, the canopy soil was shallow, suggesting plants must increase water-use efficiency in order to adapt to dry environmental conditions and low availability of soil water. Canopy plants also had high leaf N concentration and low leaf C:N ratio in relation to leaf δ^{13}C compared to ground plants. This suggested that high water-use efficiency of canopy plants may be the consequence of not only water-limitation, but also high photosynthetic capacity, as indicated by high leaf N concentration (Hikosaka et al. 1998; Takashima et al. 2004). This response in relation to leaf δ^{13}C was differed between growing habitats (canopy/ground plants) as well as among life-forms (tree, shrub, liana, herb). In ground plants, leaf N concentration increased with increasing $\delta^{13}C$, whereas C:N ratio decreased, suggesting greater water-use efficiency was achieved by investing in leaf N to enhance photosynthetic capacity. This trend was especially evident for lianas, mostly comprising *H. petiolaris* and *H. hydrangeoides*. Opposite trends for canopy plants suggested greater water-use efficiency of plants higher in the canopy was achieved by controlling transpirational water loss rather than by investing in leaf N. This trend was especially evident for trees and herbs. Because the trend observed for herbs was defined by the single, most abundant species, *D. mariesii*, further investigation is needed to determine if the trend is general for herbs or due to the behavior of this particular species.

Although $\delta^{15}N$ was not affected by growing habitat and life-form, it decreased with increasing leaf height and $\delta^{13}C$ in canopy plants, especially in ferns. The natural $\delta^{15}N$ composition of epiphytes is consistently lower than that of trees (Stewart et al. 1995) and decreases from the lower to upper canopy (Bergstrom and Tweedie 1998) suggesting that upper-canopy epiphytes mainly obtain N from atmospheric deposition, whose $\delta^{15}N$ tends to be lower than that of soil. The mean $\delta^{15}N$ of the arboreal soil (organic matter) accumulated on our study tree was – 1‰ (Tatsumi et al. in submission), while that of leaves of upper-canopy plants in this study were much lower. In addition, $\delta^{15}N$ of throughfall in this forest is higher than that of canopy plants throughout the year (Dr. K. Fukushima, personal communication). In drought-stressed plants, $\delta^{13}C$ increases and $\delta^{15}N$ decreases as a result of lower rates of carbon assimilation and reduced N demand leading to stronger ^{15}N discrimination (Robinson et al. 2000). The arboreal soils accumulated on our study tree have similar N availability as the ground soils (Tatsumi et al. 2021). Therefore, we inferred that lower $\delta^{15}N$ associated with high $\delta^{13}C$ of the canopy plants in this study may be due to water deficit in the upper canopy and not nitrogen deficiency, i.e., drought effect on ^{15}N discrimination caused by reduced photosynthesis and N demand, which has been observed in epiphytes in a tropical rainforest (Wania et al. 2002).

Conclusion

Our results show that various vascular plants utilize a single large host tree as their habitat exhibiting various ecophysiological adaptations to the unique canopy environment, and reinforcing that one large crown can function as a local hotspot for plant species diversity in a forest ecosystem. Three-dimensional structure of the large, old host tree, especially reiterated trunks, and large horizontal branches, contributed to abundance of vascular plant species in the canopy by creating habitat for them. In addition to maintaining epiphytic habitat including endangered species that can only survive in the crown of large, old trees, the accidental epiphytes found on our study trees included 14 terrestrial species affected by deer over-browsing on the forest floor. If enough host trees are conserved for these species to maintain viable populations in the canopy, the epiphytic individuals of deer-preferred species could function as future seed sources for recolonizing the forest floor. Stable isotopes analyses suggested that canopy plants, especially ferns and lianas, mitigate water-deficit by increasing water-use efficiency of leaves. Our findings reinforce the notion that ecological functions of large, old trees cannot be replaced once they are lost from an ecosystem and that large, long-lived trees should be conserved taking into account their ecological role for other organisms (Lindenmayer and Laurance 2017).

Acknowledgements We greatly appreciate the substantial contribution of Dr. Kae Takahashi, Dr. Keisuke Nishida, Mr. Takeshi Hirai, Mr. Yusaku Sasabe, and members of the Field Science, Education and Research Center (FSERC) of Kyoto University, to field survey and logistics. We also deeply thank Dr. Keisuke Koba for significant advises on support for the isotope analysis and Dr. Keitaro Fukushima for providing useful information about the study site. Isotope analysis was conducted using Cooperative Research Facilities (Isotope Ratio Mass Spectrometer) of Center for Ecological Research, Kyoto University. This study was financially supported in part by Expo '90 Foundation and Kansai Organization for Nature Conservation.

Author contributions WAA conceived the study and acquired funding. All authors conducted field studies and WAA, NK, YO conducted laboratory studies. WAA wrote the original draft, NK, YO, HI, AN edited the manuscript, and all authors reviewed the manuscript.

Declarations

Conflict of interest All authors have no conflicts of interest and confirm disclosures, declarations, and transparency on data statements.

References

Ashiu Research Forest Research Group (1987) Preserved trees and forests in the Ashiu research forest (1). Rep Kyoto Univ Forest 17:21–24

Baines D, Sage RB, Baines MM (1994) The implications of red deer grazing to ground vegetation and invertebrate communities of Scottish native pinewoods. J Appl Ecol 31:776–783. https://doi.org/10.2307/2404167

Beguin J, Tremblay JP, Thiffault N, Pothier D, Côté SD (2016) Management of forest regeneration in boreal and temperate deer-forest systems: challenges, guidelines, and research gaps. Ecosphere 7:1–16. https://doi.org/10.1002/ecs2.1488

Benzing DH (2004) Vascular epiphytes. In: Lowman MD, Rinker HB (eds) Forest canopies, 2nd edn. Elsevier, San Diego, pp 175–211

Bergstrom DM, Tweedie CE (1998) A conceptual model for integrative studies of epiphytes: nitrogen utilisation, a case study. Aust J Bot 46:273–280

Côté DS, Rooney PT, Tremblay J-P, Dussault C, Waller MD (2004) Ecological impacts of deer overabundance. Annu Rev Ecol Evol Syst 35:113–147. https://doi.org/10.2307/annurev.ecolsys.35.021103.30000006

Essen P-A, Renhorn K-E, Pettersson BR (1996) Epiphytic lichen biomass in managed and old-growth boreal forests: effect of branch quality. Ecol Appl 6:228–238

Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci 6:121–126. https://doi.org/10.1016/S1360-1385(01)01889-1

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537. https://doi.org/10.1146/annurev.pp.40.060189.002443

Fujii S (2010) Changes in the understory flora following sika deer browsing with special reference to flowering stage in Makura-dani, Ashiu exeperimental forest. Japanese J Conserv Ecol 15:3–15

Fukuda A, Takayanagi A (2008) Influence of snow cover on browsing of *Cephalotaxus harringtonia* var. *nana* by *Cervus nippon* Temminck in heavy snow region in central Japan. For Res Kyoto 77:5–11

Gorman AJ, Kerhoulas LP, Polda WT, Kerhoulas NJ (2019) Epiphyte diversity, abundance, and distribution in an old Sitka spruce crown. Evansia 36:12. https://doi.org/10.1639/0747-9859-36.1.12

Hall J (1958) How the native bromeliads took the cold in Florida. Bromel Soc Bull 8:6–7

Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ 15:965–985. https://doi.org/10.1111/j.1365-3040.1992.tb01650.x

Hattori T, Tochimoto D, Iwakiri K, Minamiyama N, Hashimoto Y (2007a) Species richness of epiphytes in a lucidophyllous forestin Kawanaka, Aya, Miyazaki Prefecture. Veg Sci 24:73–83

Hattori T, Tochimoto D, Iwakiri K, Minamiyama N, Hashimoto Y (2007b) Species richness and species composition of epiphytes in a lucidophyllous forest on Mt. Kurino-Dake, Kagoshima prefecture. Humans Nat. https://doi.org/10.24713/hitotoshizen.18.0_29

Hattori T, Tochimoto D, Noriko M, Hashimoto Y, Sawada Y, Ishida H (2009) Species richness and species compositions of epiphytes in the lucidophyllous forest in southern Kyushu. Veg Sci 26:49–61

Hikosaka K, Hanba YT, Hirose T, Terashima I (1998) Photosynthetic nitrogen-use efficiency in leaves of woody and herbaceous species. Funct Ecol 12:896–905. https://doi.org/10.1046/j.1365-2435.1998.00272.x

Hoeber V, Zotz G (2021) Not so stressful after all: epiphytic individuals of accidental epiphytes experience more favourable abiotic conditions than terrestrial conspecifics. For Ecol Manage 479:118529. https://doi.org/10.1016/j.foreco.2020.118529

Hoeber V, Weichgrebe T, Zotz G (2019) Accidental epiphytism in the Harz Mountains, Central Europe. J Veg Sci 30:765–775. https://doi.org/10.1111/jvs.12776

Hoeber V, Klinghardt M, Zotz G (2020) Drought resistance does not explain epiphytic abundance of accidental epiphytes. Plant Ecol Divers 13:175–187. https://doi.org/10.1080/17550874.2020.1729888

Hsu R, Wolf JHD (2009) Diversity and phytogeography of vascular epiphytes in a tropical-subtropical transition island, Taiwan. Flora Morphol Distrib Funct Ecol Plants 204:612–627. https://doi.org/10.1016/j.flora.2008.08.002

Ishii HR, Minamino T, Azuma W, Hotta K, Nakanishi A (2018) Large, retained trees of *Cryptomeria japonica* functioned as refugia for canopy woody plants after logging 350 years ago in Yakushima, Japan. For Ecol Manage 409:457–467. https://doi.org/10.1016/j.foreco.2017.11.034

Kartzinel TR, Trapnell DW, Shefferson RP (2013) Critical importance of large native trees for conservation of a rare neotropical epiphyte. J Ecol 101:1429–1438. https://doi.org/10.1111/1365-2745.12145

Kato M, Okuyama Y (2004) Changes in the biodiversity of a deciduous forest ecosystem caused by an increase in the sika deer population at Ashiu, Japan. Contrib from Biol Lab Kyoto Univ 29:437–448

Kondo D, Kato S, Komiyama A (2008) Distribution and species richness of vascular epiphytes in an old-growth beech forest. Jpn Soc Forest Environ 50:9–16. https://doi.org/10.18922/jjfe.50.1_9

Kramer RD, Sillett SC, Van Pelt R (2018) Quantifying aboveground components of *Picea sitchensis* for allometric comparisons among tall conifers in North American rainforests. For Ecol Manage 430:59–77. https://doi.org/10.1016/j.foreco.2018.07.039

Kreft H, Köster N, Küper W, Nieder J, Barthlott W (2004) Diversity and biogeography of vascular epiphytes in western Amazonia, Yasuní, Ecuador. J Biogeogr 31:1463–1476. https://doi.org/10.1111/j.1365-2699.2004.01083.x

Krömer T, Kessler M, Robbert Gradstein S, Acebey A (2005) Diversity patterns of vascular epiphytes along an elevational gradient in the Andes. J Biogeogr 32:1799–1809. https://doi.org/10.1111/j.1365-2699.2005.01318.x

Kubo M, Sakio H (2020) *Cercidiphyllum japonicum*. In: Sakio H (ed) Long-term ecosystem changes in Riparian forests. Springer, Singapore, pp 55–82

Kyoto Prefecture (2015) Red data book of Kyoto prefecture. http://www.pref.kyoto.jp/kankyo/rdb/index.html. Accessed 21 Aug 2020

Laube S, Zotz G (2003) Which abiotic factors limit vegetative growth in a vascular epiphyte? Funct Ecol 17:598–604. https://doi.org/10.1046/j.1365-2435.2003.00760.x

Lie MH, Arup U, Grytnes JA, Ohlson M (2009) The importance of host tree age, size and growth rate as determinants of epiphytic lichen diversity in boreal spruce forests. Biodivers Conserv 18:3579–3596. https://doi.org/10.1007/s10531-009-9661-z

Lindenmayer DB, Laurance WF (2017) The ecology, distribution, conservation and management of large old trees. Biol Rev 92:1434–1458. https://doi.org/10.1111/brv.12290

Lindenmayer DB, Laurance WF, Franklin JF (2012) Global decline in large old trees. Science 338:1305–1306

McCune B, Amsberry KA, Camacho FJ, Clery S, Cole C, Emerson C, Felder G, French P, Greene D, Harris R, Hutten M, Larson B, Lesko M, Majors S, Markwell T, Parker GG, Pendergrass K, Peterson EB, Peterson ET, Platt J, Proctor J, Rambo T, Rosso A, Shaw D, Turner R, Widmer M (1997) Vertical profile of epiphytes in a Pacific Northwest old-growth forest. Northwest Sci 71:145–152

Miller JED, Villella J, Carey G, Carlberg T, Root HT (2017) Canopy distribution and survey detectability of a rare old-growth forest lichen. For Ecol Manage 392:195–201. https://doi.org/10.1016/j.foreco.2017.03.007

Ministry of the Environment Government of Japan (2014) The 4th version of the Japanese red lists on 9 taxonomic groups. https://ikilog.biodic.go.jp/Rdb/env. Accessed 21 Aug 2020

Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit JM, Barbaroux C, Le Thiec D, Bréchet C, Brignolas F (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. New Phytol 169:765–777. https://doi.org/10.1111/j.1469-8137.2005.01630.x

Nadkarni NM, Merwin MC, Nieder J (2001) Forest canopies, plant diversity. Encycl Biodivers Second Ed 3:516–527. https://doi.org/10.1016/B978-0-12-384719-5.00158-1

Nadkarni NM, Schaeferz D, Matelson TJ, Solano R (2002) Comparison of arboreal and terrestrial soil characteristics in a lower montane forest, Monteverde, Costa Rica. Pedobiologia 33:24–33

Nakanishi A, Sungpalee W, Sri-ngernyuang K, Kanzaki M (2013) Determination of epiphyte biomass composition and distribution with a three-dimensional mapping method in a tropical montane forest in northern Thailand. Tropics 22:27–37. https://doi.org/10.3759/tropics.22.27

Nakanishi A, Sungpalee W, Sri-ngernyuang K, Kanzaki M (2016) Large variations in composition and spatial distribution of epiphyte biomass on large trees in a tropical montane forest of northern Thailand. Plant Ecol 217:1157–1169. https://doi.org/10.1007/s11258-016-0640-7

Ortega-Solís G, Díaz I, Mellado-Mansilla D, Moreno-González R, Godoy J, Samaniego H (2020) The importance of tree species and size for the epiphytic bromeliad *Fascicularia bicolor* in a south-american temperate rainforest (Chile). Iforest 13:92–97. https://doi.org/10.3832/ifor2710-013

Parker GG (1995) Structure and microclimate of forest canopies. In: Lowman MD, Nadkarnil NM (eds) Forest canopies. Academic Press, San Diego, pp 73–106

R Development Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Robinson D, Handley LL, Scrimgeour CM, Gordon DC, Forster BP, Ellis RP (2000) Using stable isotope natural abundances (δ^{15}N and δ^{13}C) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. J Exp Bot 51:41–50. https://doi.org/10.1093/jxb/51.342.41

Rooney TP, McCormick RJ, Solheim SL, Waller DM (2000) Regional variation in recruitment of hemlock seedlings and saplings in the upper Great Lakes, USA. Ecol Appl 10:1119–1132. https://doi.org/10.1890/1051-0761(2000)010[1119:RVIROH]2.0.CO;2

Sakaguchi S, Fujiki D, Inoue M, Takayanagi A (2008) Plant species diversity and community structure of old growth-beech forest in Kamitani, Ashiu, Kyoto -community strctureand endangered plant species detected by gradsect networks-. Forest Res Kyoto 77:43–61

Sakaguchi S, Fujiki D, Inoue M, Yamasaki M, Fukushima K, Takayanagi A (2012) The effects of deer browsing on forest regeneration processes and diversity of tree communities in a mountainous region with heavy snowfall of Central Japan. Forest Res Kyoto 78:57–69

Sakaguchi S, Fujiki D, Inoue M, Yamasaki M, Fukushima K, Takayanagi A (2012b) Plant species preference of sika deer in cool-temperate mixed conifer-broadleaf forest of the sea of Japan side of Central Japan. Forest Res Kyoto 78:71–80

Seto M, Higa M, Ishikawa S (2020) Host size preferences of vascular epiphytes are reflected in their spatial distributions: a study of a mature broadleaf evergreen forest in Kochi, Japan. J Forest Res 25:358–363. https://doi.org/10.1080/13416979.2020.1779909

Shaw DC (2004) Vertical organization of canopy biota. In: Lowman MD, Rinker HB (eds) Forest canopies, 2nd edn. Elsevier, San Diego, pp 73–101

Sillett SC, Antoine ME (2004) Lichens and bryophytes in forest canopies. In: Lowman MD, Rinker HB (eds) Forest canopies, 2nd edn. Elsevier, San Diego, pp 151–174

Sillett SC, Van Pelt R (2007) Trunk reiteration promotes epiphytes and water storage in an old-growth redwood forest canopy. Ecol Monogr 77:335–359. https://doi.org/10.1890/06-0994.1

Soetaert K (2017) Plotting multi-dimensional data. R package version 1.1.1. https://cran.r-project.org/web/packages/plot3D/index.html. Accessed 5 Jan 2018

Stewart GR, Schmidt S, Handley LL, Turnbull MHH, Erskine PD, Joly CAA (1995) ^{15}N natural abundance of vascular rainforest epiphytes: implications for nitrogen source and acquisition. Plant Cell Environ 18:85–90. https://doi.org/10.1111/j.1365-3040.1995.tb00547.x

Takashima T, Hikosaka K, Hirose T (2004) Photosynthesis or persistence: Nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. Plant Cell Environ

27:1047–1054. https://doi.org/10.1111/j.1365-3040.2004.01209.x

Takatsuki S (2009) Effects of sika deer on vegetation in Japan: a review. Biol Conserv 142:1922–1929. https://doi.org/10.1016/j.biocon.2009.02.011

Tamura A (2016) Potential of soil seed banks in the ecological restoration of overgrazed floor vegetation in a cool-temperate old-growth damp forest in eastern Japan. J for Res 21:43–56. https://doi.org/10.1007/s10310-015-0509-y

Tatsumi C, Azuma WA, Ogawa Y, Komada N (2021) Nitrogen availability and microbial communities of canopy soils in a large *Cercidiphyllum japonicum* tree of a cool-temperate old growth forest. Microb Ecol. https://doi.org/10.1007/s00248-021-01707-w

Tochimoto D, Hattori T, Iwakiri K, Minamiyama N, Sawada Y (2008) Species richness and species compositions of epiphytes in a lucidophyllous forest in Mt. Omoridake, Aya, Miyazaki prefecture. Veg Sci 25:63–72

Wania R, Hietz P, Wanek W (2002) Natural ^{15}N abundance of epiphytes depends on the position within the forest canopy: source signals and isotope fractionation. Plant Cell Environ 25:581–589. https://doi.org/10.1046/j.1365-3040.2002.00836.x

Williams CB, Sillett SC (2007) Epiphyte communities on redwood (*Sequoia sempervirens*) in Northwestern California. Bryologist 110:420–452

Woods CL, Cardelús CL, DeWalt SJ (2015) Microhabitat associations of vascular epiphytes in a wet tropical forest canopy. J Ecol 103:421–430. https://doi.org/10.1111/1365-2745.12357

Yamanaka N, Matsumoto A, Oshima Y, Kawanabe S (1993) Stand structure of Mondori-Dani watershed Kyoto university forest in Ashiu. Rep Kyoto Univ Forest 65:63–76

Zotz G (2005) Vascular epiphytes in the temperate zones—a review. Plant Ecol 176:173–183. https://doi.org/10.1007/s11258-004-0066-5

Zotz G (2013a) Hemiepiphyte: a confusing term and its history. Ann Bot 111:1015–1020. https://doi.org/10.1093/aob/mct085

Zotz G (2013b) The systematic distribution of vascular epiphytes-a critical update. Bot J Linn Soc 171:453–481. https://doi.org/10.1111/boj.12010