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

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Abstract

Large trees have survived for a long time, and their complex crown structure serves 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, over-browsing), promoting and conserving forest species diversity. Terrestrial species may also vary in their ability to adapt to 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 with 39 vascular plant species, including 31 accidental epiphytes and six threatened, obligate epiphytes. High numbers of canopy plant species, including most accidental epiphytes, were found where multiple, large reiterated trunks as well as on large horizontal branches. Canopy plants leaves exhibited higher water use efficiency and higher nitrogen concentration compared to plants on the ground, but were controlled by transpirational water loss rather than by investing in leaf nitrogen with increasing height. We found at least 14 species could escape over-browsing by establishing in the canopy and may function as seed-sources for future re-colonization on the ground. 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.

1. 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 or epiphytes are found in various climatic zones. For example, in tropical montane or rain forests, vascular epiphytes especially orchids and bromeliads 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; Ortega-Solís et al. 2020). 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 by directly accessing the canopy using rope-climbing techniques (e.g. McCune et al. 1997; Williams et al. 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 by 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). Although accidental epiphytes are not the main focus of typical epiphyte studies, the canopy environment is important for maintaining species diversity in old-growth forest by providing growing conditions not available on the forest floor, e.g., high light intensity necessary for survival of shade-intolerant species (Ishii et al. 2018). Microclimatic conditions such as increasing light intensity, wind speed, and temperature change vertically from the ground to the outer layer of the canopy (Parker 1995; Shaw 2004). This can be both beneficial and limiting for establishment of terrestrial species. Water, especially, can be a major limiting factor for growth and survival of epiphytes (Laube and Zotz 2003; Hoeber et al. 2019). Accidental epiphytes are often found in wet forests growing on large, old trees with accumulations of organic material (arboreal soil, *sensu* Nadkarni et al. 2002, Enloe et al 2006). However, drought-tolerance per se, does not seem to explain which species become accidental epiphytes (Hoeber et al. 2020). Being able to survive both on the ground and in the canopy, would contribute to persistence of plant species in old-growth forest. Thus, some terrestrial species may have acquired ecophysiological characteristics that allow them to adapt to both growing conditions.

Here, we investigated species composition and ecophysiological characteristics of canopy vascular plants hosted on the crown of a large *Cercidiphyllum japonicum* tree in a temperate old-growth forest, Japan. This tree shows a prominently developed tree architecture with abundant arboreal soil, which have similar nitrogen availability as terrestrial soil (Tatsumi et al. in submission). To examine epiphyte diversity in relation to host tree structure and environmental conditions, we documented the three-dimensional architecture of the host tree and vertical temperature gradient. All canopy measurements were conducted using rope climbing techniques that are highly reliable for direct observation of epiphytes (Miller et al. 2017). To compare ecophysiological characteristics between plants growing in the canopy and on the ground and infer their water- and nutrient-use efficiencies, we measured in leaf traits such as carbon (C) and nitrogen (N) concentrations and stable isotope compositions of leaves at various heights of plants in the crown of the host tree and on the ground. Our objective was to evaluate the ecological value of large, old trees for purposes of conservation of vascular plant diversity.

2. Materials And Methods

2.1. 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 to 959 m a.s.l.). This forest is located in the transition zone between cool temperate forests and warm temperate forests and more than half of it is covered with old-growth forest. There is a heavy snowfall ranging from 1 to 3 m in winter. The mean monthly temperature ranged from − 0.4°C in January to 24.0°C in August and the mean annual precipitation was 2,568 mm at the forest weather station at 350 m a.s.l. from 2011 to 2015. In this forest, over-browsing by deer had serious negative impact on recruitment and diversity of understory vegetation, as well as on the animal and insect fauna that utilize it (Fujii, 2010; Fukuda and Takayanagi, 2008; Kato and Okuyama, 2004; Sakaguchi et al., 2008).

Our study tree (host tree) was a *Cercidiphyllum japonicum* Siebold & Zucc. (38 m tall and ca. 3.5 m of the diameter at breast height) which was a one of the largest trees in the forest reserve. It is located at 640 m a.s.l. in a cool temperate old-growth forest. We accessed the crown non-destructively using single and double-rope climbing technique for the investigation described below.

2.2 Measurement of tree architecture and vertical temperature variation in the host tree

To reconstruct three-dimensional structure of the host tree, the tree architecture was mapped in three-dimensional coordinate space by using a hand-held laser range finder (TruePalse360, Laser Technology, Inc., USA) according to 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: the base of the trunk, nodes, and ends of branches (see Kramer et al. (2018) for definition of measured elements). 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 considered as an aggregate of these segments.

From June to August 2017, vertical temperature changes within crown was measured at hourly interval using thermal sensors (Thermochron Type-G, KN Laboratories, Inc., Japan) which were installed on the crown of the host tree at 0, 5, 10, 15, 20, 26, 30, and 33 m above ground height.

2.3 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 its species name and the height of its stem base. Lianas were recorded at the height where leaves appeared, because it was difficult to determine the rooting point and distinguish individual plants.

2.4 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 45 leaves of canopy plants and 17 leaves of ground plants (including five leaves of host tree sampled from various heights) in August 2017 and June 2018. The sampled leaves represented 10 tree species (excluding the host tree), three shrub species, three herbaceous species (including two ferns), and four liana species (Online Resource 1).

After collection, the sample leaves were dried at 60 °C, ground to fine powder, and loaded into capsules for isotope analysis. Measurements of total N and C concentrations and stable isotope ratios were done 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: $\delta^{15}N$ or $\delta^{13}C$ = [Rsample/Rstandard-1] × 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. Generally, $\delta^{15}N$ of plant tissue reflects isotope fractionation during N uptake and N assimilation, as well as $\delta^{15}N$ of the N sources (Handley and Raven 1992; Evans 2001). The $\delta^{15}N$ of soil tends to be higher than that of N deposition from the atmosphere (Ohte et al. 2008). While $\delta^{13}C$ increases with decreasing stomatal conductance and can be used as a measure of leaf water stress (Koch et al. 2004).

2.5 Data analysis

Three-dimensional architecture of the host tree was depicted using R Package 'plot 3D' 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.

Canopy plant species were categorized according to life-form (trees, shrubs, lianas, and herbs), threatened/non-threated species, accidental/obligate epiphytes, deer preference, 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.* (2012), which investigated the effect of deer browsing in the Ashiu Forest Reserve.

Leaf ecophysiological characteristics were analyzed in relation to leaf height and leaf $\delta^{13}C$ using regression analysis. The relationships were analyzed in an analysis of covariance (ANCOVA) to test effects of growing habitat (canopy and ground) and life-form (trees, shrubs, lianas, and herbs). We used JMP14 (SAS Institute, Cary, NC, USA) for all analyses.

3. Results

3.1 Tree architecture and vertical temperature variation

The three-dimensionally reconstructed architecture of the host tree as viewed facing north, south, east, 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 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 (Table 1). 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 branches increased above ca. 20 m.

Table 1 Distribution of each height of the segments, which are the elements of the three-dimensionally reconstructed architecture of the host tree. The column represents the number of segments in each inclination angle.

Height (m)	Inclination of the segments (˚)									
	-45	-30	-15	0	15	30	45	60	75	90
30~35	0	0	0	0	0	2	1	2	1	2
25~30	0	0	0	1	5	5	7	6	6	4
20~25	0	0	0	6	13	10	4	2	4	3
15~20	0	0	0	2	5	5	3	3	5	0
10~15	1	0	0	0	2	0	3	1	2	3
5~10	0	0	0	1	0	0	1	4	2	2
0~5	0	0	0	1	0	0	0	0	0	1

The mean temperature and the mean daily temperature difference increased with height (Fig. 2). The mean temperature ranged from 20.5 °C at 0 m to 21.5 °C at 20, 26, 30, 33 m. Mean temperature was highest and daily temperature difference was largest at heights ranging 25 – 35 m and decreased at the lower canopy.

3.2 Canopy plant community

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

Table 2 List of canopy plants found on the study tree (*Cercidiphyllum japonicum*) with information of life-form, threatened species, accidental epiphytes, deer preference, and the height where individuals were found. For threatened species, check mark indicates a species listed in the Kyoto Red Data Book, while species listed in the Japan Red List are indicated by the Red List categories (NT: Near Threatened, VU: Vulnerable, EN: Endanger). The fill colors show the heights of individual canopy plants were sorted at 5 m intervals from the ground (0 m) according to the height of their stem base.

Families	Species	Life-form	Threatened species	Accidental epiphytes	Deer preferences	Height (m)					
						0~5	5~10	10~15	15~20	20~25	25~30
Anacardiaceae	*Rhus javanica* L. var. *chinensis* (Mill.) T.Yamaz.	Trees		✓	✓						
Anacardiaceae	*Toxicodendron trichocarpum* (Miq.) Kuntze	Trees		✓							
Aquifoliaceae	*Ilex macropoda* Miq.	Trees		✓	✓						
Araliaceae	*Chengiopanax sciadophylloides* (Franch. et Sav.) C.B.Shang et J.Y.Huang	Trees		✓							
Araliaceae	*Aralia elata* (Miq.) Seem.	Trees		✓	✓						
Rosaceae	*Aria alnifolia* (Siebold & Zucc.) Decne.	Trees		✓							
Rosaceae	*Padus grayana* (Maxim.) C.K.Schneid.	Trees		✓							
Rosaceae	*Sorbus commixta* Hedl.	Trees		✓							
Rosaceae	*Cerasus jamasakura* (Siebold ex Koidz.) H.Ohba	Trees		✓							
Sapindaceae	*Acer rufinerve* Siebold et Zucc.	Trees		✓	✓						
Sapindaceae	*Acer amoenum* Carrière	Trees		✓							
Trochodendraceae	*Trochodendron aralioides* Siebold et Zucc.	Trees		✓							
Alnaceae	*Corylus sieboldiana* Blume	Shrubs		✓	✓						
Aquifoliaceae	*Ilex serrata* Thunb.	Shrubs		✓							
Celastraceae	*Euonymus alatus* (Thunb.) Siebold var. *alatus* f. *striatus* (Thunb.) Makino	Shrubs		✓	✓						
Celastraceae	*Euonymus macropterus* Rupr.	Shrubs		✓							
Clethraceae	*Clethra barbinervis* Siebold et Zucc.	Shrubs		✓	✓						
Ericaceae	*Vaccinium smallii* A. Gray var. *versicolor* (Koidz.) T.Yamaz.	Shrubs		✓							
Ericaceae	*Lyonia ovalifolia* (Wall.) Drude var. *elliptica* (Siebold et Zucc.) Hand.-Mazz.	Shrubs		✓	✓						
Grossulariaceae	*Ribes ambiguum* Maxim.	Shrubs	✓, NT								
Helwingiaceae	*Helwingia japonica* (Thunb.) F.Dietr.	Shrubs		✓							
Lamiaceae	*Callicarpa japonica* Thunb.	Shrubs		✓	✓						
Taxaceae	*Cephalotaxus harringtonia* (Knight ex Forbes) K.Koch var. *nana* (Nakai) Rehder	Shrubs		✓	✓						
Thymelaeaceae	*Daphne miyabeana* Makino	Shrubs	✓	✓							
Actinidiaceae	*Actinidia polygama* (Siebold et Zucc.) Planch. ex Maxim.	Lianas		✓	✓						
Celastraceae	*Celastrus orbiculatus* Thunb. var. *strigillosus* (Nakai) H.Hara	Lianas		✓							
Hydrangeaceae	*Hydrangea hydrangeoides* (Siebold et Zucc.) B.Schulz	Lianas		✓							
Hydrangeaceae	*Hydrangea petiolaris* Siebold et Zucc.	Lianas		✓							
Caryophyllaceae	*Stellaria diversiflora* Maxim.	Herbs		✓	✓						
Davalliaceae	*Davallia mariesii* T.Moore ex Baker	Herbs									
Dryopteridaceae	*Dryopteris crassirhizoma* Nakai	Herbs		✓	✓						
Huperzia	*Huperzia cryptomerina* (Maxim.) Dixit	Herbs	✓, VU								
Hymenophyllaceae	*Crepidomanes minutum* (Blume) K.Iwats.	Herbs									
Orchidaceae	*Goodyera pendula* Maxim.	Herbs	✓, EN								
Orchidaceae	*Hemipilia chidori* (Makino) Y.Tang, H.Peng et T.Yukawa	Herbs	✓, VU								
Oxalidaceae	*Oxalis griffithii* Edgew. et Hook.f.	Herbs		✓							

Polypodiaceae	*Polypodium fauriei* Christ	Herbs											
Polypodiaceae	*Lepisorus annuifrons* (Makino) Ching	Herbs	✓										
Urticaceae	*Laportea cuspidata* (Wedd.) Friis	Herbs		✓									

The canopy plants showed wide vertical distribution on the host tree, ranging in height from the trunk base to 28 m (Table 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 more than 15 m height difference between highest and lowest observed individuals.

3.3 Leaf ecophysiological characteristics of canopy plants

Figure 3 and 4 show the relationship between each leaf ecological characteristic and leaf height and leaf $\delta^{13}C$ (an index of leaf water stress), organized by growing habitat and life-form. The results of regression analysis were shown in Table 3.

Table 3 Results of regression analysis of leaf ecophysiological characteristics in relation to leaf height and leaf $\delta^{13}C$.

Dependent variable		$\delta^{13}C$		N content				C:N ratio				$\delta^{15}N$			
Independent variable		leaf height		leaf height		$\delta^{13}C$		leaf height		$\delta^{13}C$		leaf height		$\delta^{13}C$	
		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	< 0.001	0.31	< 0.001	0.30	< 0.001	0.31	< 0.001	0.31	< 0.001	0.20	**0.001**	0.31	< 0.001
	ground (n = 17)	0.19	< 0.05	0.10	0.11	0.67	< 0.001	0.02	0.28	0.63	< 0.001	0.03	0.23	0.04	0.43
Plant life-form	tree (n = 18)	0.14	0.07	0.07	0.30	0.44	< 0.01	0.03	0.51	0.47	**0.001**	0.002	0.88	0.15	0.07
	shrub (n = 3)	0.25	0.66	0.52	0.46	0.05	0.85	0.35	0.60	0.16	0.74	0.30	0.62	0.20	0.71
	liana (n = 18)	0.31	< 0.01	0.004	0.80	0.17	**0.05**	0.04	0.44	0.35	< 0.01	0.16	0.06	0.01	0.70
	herb (n = 17)	0.63	< 0.001	0.26	< 0.05	0.26	< 0.05	0.22	< 0.05	0.24	< 0.05	0.18	**0.05**	0.15	0.07
	host tree (n = 5)	0.14	0.29	0.43	0.14	0.05	0.71	0.78	< 0.05	0.15	0.52	0.32	0.19	0.20	0.45

By growing habitat, leaf $\delta^{13}C$ increased with leaf height for both canopy plants and ground plants (Fig. 3a). For the canopy plants, leaf N concentration (Fig. 3b) and leaf $\delta^{15}N$ (Fig. 3c) decreased, while leaf C:N ratio (Fig. 3d) increased with leaf height, whereas for the ground plants, these variables did not vary with leaf height. Trends observed for leaf traits in relation to leaf $\delta^{13}C$ were opposite for canopy and ground plants as indicated by significant interaction terms for ANCOVA (Fig. 3, Online Resource 2). For example, leaf N concentration decreased with increasing leaf $\delta^{13}C$ for the canopy plants, whereas it increased for the ground plants (Fig. 3e) There was no significant effects of growing habitat on leaf $\delta^{13}C$ and leaf $\delta^{15}N$ 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. 3, Online Resource 2). There was no effect of growing habitat on leaf $\delta^{15}N$ in relation to leaf $\delta^{13}C$, while leaf N concentration was higher and leaf C:N ratio was lower in canopy plants compared to ground plants (Fig. 3, Online Resource 2).

Leaf ecophysiological characteristics also varied by life-form. Leaf $\delta^{13}C$ increased with leaf height in lianas and herbs (Fig. 4a), while leaf N concentration and leaf $\delta^{15}N$ decreased in herbs (Fig. 4b), and leaf C:N ratio increased in herbs and host tree (Fig. 4c). Trends observed for leaf traits in relation to leaf $\delta^{13}C$ were opposite in lianas and the others as indicated by significant interaction terms for ANCOVA (Fig. 4, Online Resource 2). For example, leaf N concentration decreased with increasing leaf $\delta^{13}C$ in trees and herbs, whereas it increased in lianas (Fig. 4e). Leaf C:N ratio increased with increasing leaf $\delta^{13}C$ in herbs and trees, whereas it decreased in lianas (Fig. 4f). There was no significant effect of life-form on leaf $\delta^{15}N$ in relation to leaf height, while significant effects were observed for leaf $\delta^{13}C$, leaf N concentration, and leaf C:N ratio (Fig. 4, Online Resource 2). There was no effect of life-form on the relationship of leaf N concentration, leaf C:N ratio, and leaf $\delta^{15}N$ with leaf $\delta^{13}C$ (Fig. 4, Online Resource 2).

4. Discussion

4.1 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. 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 contribute to changes in epiphyte community structure (Sillett and Antoine 2004; Woods et al. 2015). Globally, however, large trees and old-growth forests are scarce, which could seriously affect the long-term conservation of endemic 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.

On the other hand, nearly 80% of canopy vascular plant species hosted 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 Europe, Chile, New Zealand, and 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 evergreen (lucidophyllous) natural forests in southern Japan (Hattori et al. 2007a; Hattori et al. 2007b; Tochimoto et al. 2008; Hattori et al. 2009; 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 at 1050 m elevation in Yakushima Island, southern Japan dominated by *Cryptomeria japonica* (Ishii et al. 2018). These observations suggest that obligate epiphytes decrease in cooler climates, because lower temperatures are a major factor limiting the distribution of tropical (lowland) epiphyte taxa (Hall 1958; Zotz 2005). However, it was suggested that vascular epiphytes in Central European are not necessarily limited by low temperatures, but by the lack of host tree structures needed for establishments (Hoeber and Zotz 2021). Large, old trees, important structural features of old-growth forest (Franklin et al. 2018) upon which epiphytes could potentially establish, are scarce in temperate forests of heavily populated regions like Europe.

Host tree structures, such as large surface area, contribute to the occurrence of mat-like canopy plant communities (Nakanishi et al. 2013; Nakanishi et al. 2016). Size and age of trunks and branches contribute to accumulation of canopy soils, a regulating factor for size and abundance of canopy plants (Sillett and Van Pelt 2007; Ishii et al. 2018). In this study, high numbers of canopy plant species, including most accidental-epiphyte tree species, were found where multiple, large reiterated trunks as well as on large horizontal branches of the host tree. In addition, canopy soils with similar nitrogen availability to ground soils were found (Tatsumi et al. in submission), suggesting long-term accumulation of organic matter contributes to establishment of terrestrial species that originally take root on the forest floor. 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 function as an important refuge for deer-preferred species to escape over browsing. Effect of excessive deer foraging on vegetation is a serious issue in various forest ecosystems (e.g., Côté et al. 2004; Beguin et al. 2016) including Japan (Takatsuki 2009). The pronounced negative effect impact of over browsing on regeneration and establishment of deer-preferred plant 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 rarely grow to reproductive size in the canopy (Zotz 2013). In the canopy of large *Cryptomeria japonica* trees in Yakushima Island, Japan, however, several accidental epiphyte species reproduced and maintained viable populations (Ishii et al. 2018). In our study tree, some woody species grew larger than 5 m in height and several species were observed to flower and fruit every year. Large, old trees are not only function as a temporary refuge for the deer-preferred species, they can also be expected to be seed sources for future recolonization on the forest floor.

4.2 Canopy plant ecophysiology

Our analyses of stable isotope composition and nitrogen concentration of leaves suggested that leaf water-use efficiency increased with increasing height and the canopy plant realized high nitrogen concentration compared to ground plants. 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 $\delta^{13}C$ (Monclus et al. 2006).

Increasing leaf $\delta^{13}C$ with height in both the ground and canopy plants reflect acclimation to increasing water deficit from the forest floor to the canopy. As leaves of the host tree showed similar $\delta^{13}C$ values regardless of height, the trend among canopy plants may reflect acclimation at the individual, interspecies or life-form scale. Ferns, which were mostly distributed in the upper-most part of the crown (20–30 m) showed particularly high leaf $\delta^{13}C$ values. Temperature was highest and daily temperature difference most variable in the upper-most crown. In addition, the canopy soil was thin, suggesting plants must increase water-use efficiency in order to adapt to harsh environmental conditions.

Canopy plants also had high leaf N concentration and low leaf C:N ratio in relate to leaf $\delta^{13}C$ compared to ground plants. This suggested high water use efficiency of canopy plants was affected by not only water limiting on the canopy but also by the high photosynthetic capacity related to the higher leaf N concentration (Hikosaka et al. 1998; Takashima et al. 2004). However, the response with increasing leaf $\delta^{13}C$ was different by growing habitat (canopy /ground plants) and life-form (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 is achieved by investing in leaf N to enhance photosynthetic capacity. This trend was especially evident for lianas. Opposite trends for canopy plants suggest 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.

Although $\delta^{15}N$ was not affected by growing habitat and life-form, it decreased with leaf height and $\delta^{13}C$ in canopy plants. 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 our study tree was − 1‰ (Tatsumi et al. in submission), while that of leaves of canopy plants in the upper canopy were much lower in this study. 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). Based on these observations, we inferred that 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). 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). In this study, because the arboreal soils accumulated our study tree had similar N availability as the ground soils (Tatsumi et al. in submission), lower $\delta^{15}N$ associated with high $\delta^{13}C$ of the canopy plants may be due to water deficit in the upper canopy and not nitrogen deficiency.

5. Conclusion

Our results show that various vascular plants utilize a single large host tree as their habitat while acclimating to the unique canopy environment, suggesting one large crown can function as a local hotspot for plant species diversity in a forest ecosystem. In addition to maintaining habitat for endemic epiphytes including endangered species that can only survive in the crown of large, old trees, the many accidental epiphyte species found temporary refuge from deer browsing in our study tree. The epiphytic individuals of deer-preferred species will function as future seed sources for recolonizing the forest floor, suggesting that the ability of accidental epiphytes to survive in the canopy may be adaptive for avoiding local extinction due to over browsing. We found various leaf traits that allow vascular plants to adapt to the canopy environment at the individual, interspecies or life-form scale. 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).

Declarations

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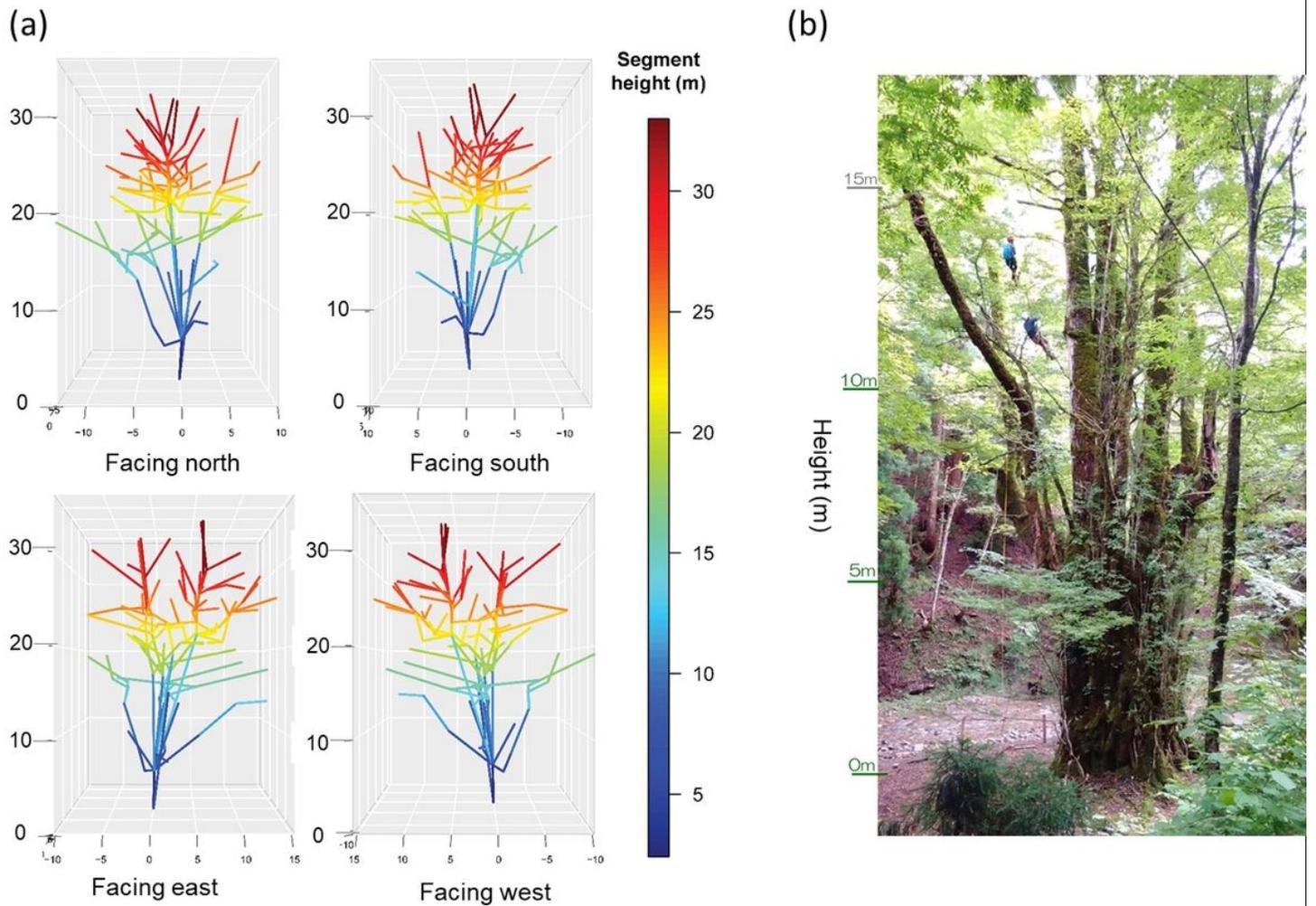
Figures

Figure 1

Three-dimensionally reconstructed architecture of the study trees as viewed facing north, south, east, and west drawn using R Package 'plot 3D'. Color bars indicate the height of each segment reconstructing the host tree. (b) The photo is closest to the view facing south. Note climbers on rope in the photo.
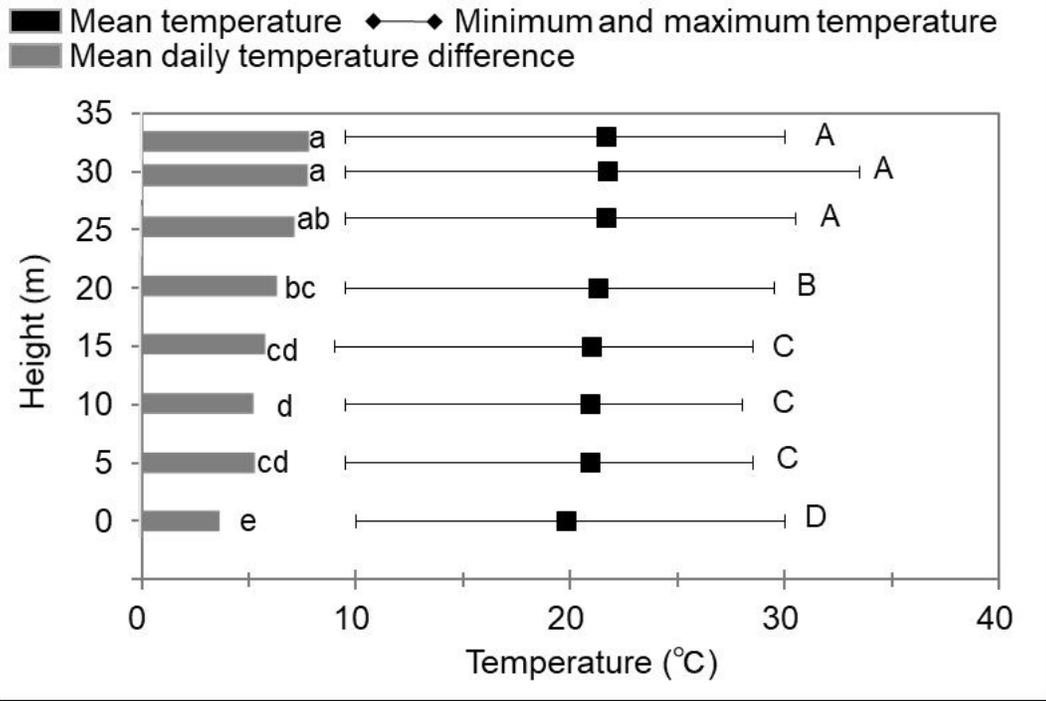
Figure 2

Vertical variation of mean, minimum, maximum temperatures and the mean daily temperature difference in relation to height in the host tree from June to August 2017. 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).
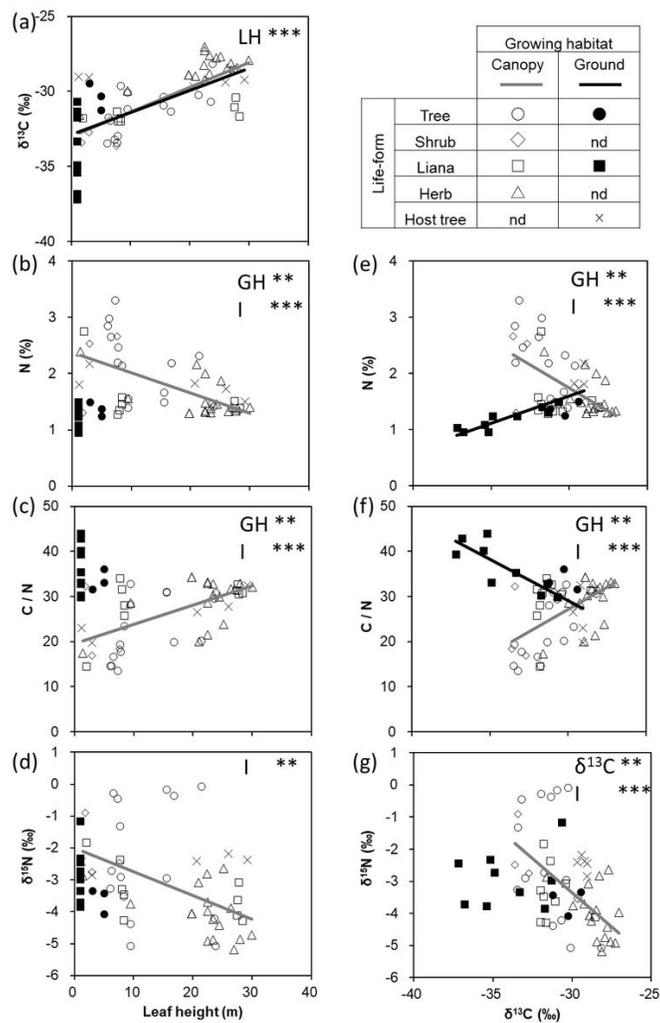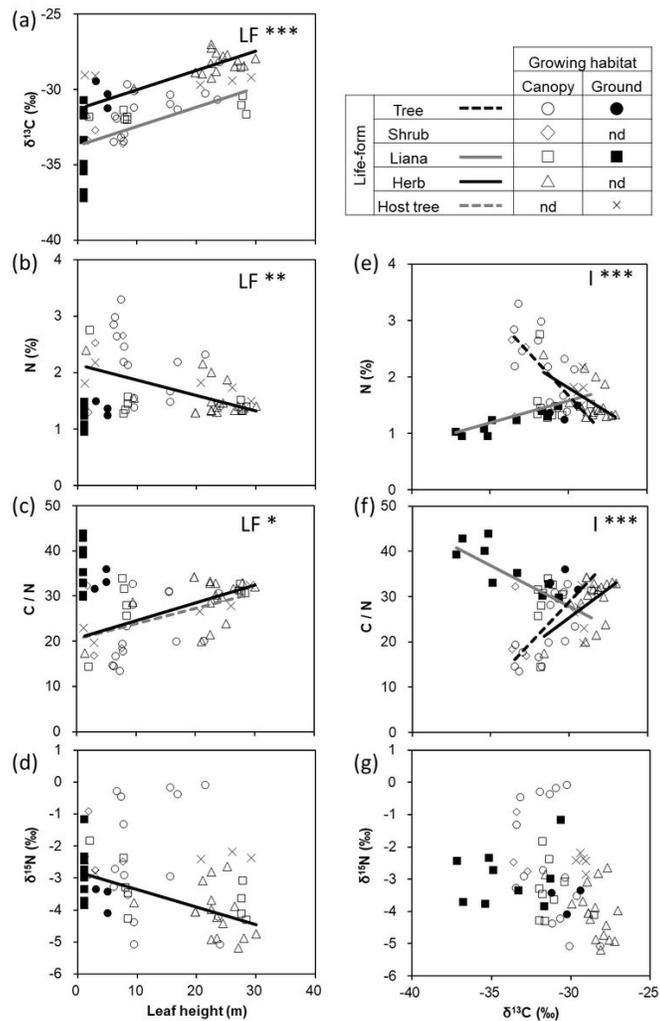
Figure 3

The comparison between growing habitat of canopy plants (open symbols) and ground plants (closed symbols) on the relationship between leaf compositional deviation of 13C (δ13C), leaf nitrogen concentration (N), leaf C:N ratio, and compositional deviation of 15N (δ15N) with increasing (a-d) leaf height and (e-g) leaf δ13C. Gray and black solid line indicate significant linear regression for canopy plants and ground plants, respectively (See Table 3). Symbol shapes indicate plant life-forms (see legend). The right side of each panels shows the p-values (*p < 0.05, **p < 0.01, ***p < 0.001) based on the ANCOVA with leaf height (LH) and leaf δ13C (δ13C) as the main effect, growing habitat (GH) as covariates, and their interaction (I). See Online Resource 2 for the detail results of ANCOVA.

Figure 4

The comparison between life-form of tree (circle), shrub (diamond), liana (square), herb (triangle), and host tree (cross) on the relationship between leaf compositional deviation of 13C (δ13C), leaf nitrogen concentration (N), leaf C:N ratio, and compositional deviation of 15N (δ15N) with increasing (a-d) leaf height and (e-g) leaf δ13C. Gray and black solid line indicate significant linear regression for lianas and herbs, respectively (See Table 3). Gray and black dotted line indicate significant linear regression for host tree and trees, respectively (See Table 3). Open and closed symbol indicate canopy plants and ground plants, respectively. The right side of each panels shows the p-values (*p < 0.05, ** p < 0.01, *** p < 0.001) based on the ANCOVA with leaf height (LH) and leaf δ13C (δ13C) as the main effect, life-form (LF) as covariates, and their interaction (I). See Online Resource 2 for the detail results of ANCOVA.

Supplementary Files

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