

Microhabitat associations of vascular epiphytes in a wet tropical forest canopy

Carrie L. Woods^{1*}, Catherine L. Cardelús¹ and Saara J. DeWalt²

¹Department of Biology, Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA; and ²Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA

Summary

1. In tropical forests, vascular epiphyte diversity increases with tree size, which could result from an increase in area, time for colonization or an increase in microhabitat heterogeneity within-tree crowns if vascular epiphyte species are specialized to particular microhabitats within the crown. The importance of microhabitats in structuring epiphyte communities has been hypothesized for more than 120 years but not yet confirmed.

2. We tested the importance of microhabitats in structuring epiphyte communities by examining microhabitat heterogeneity and epiphyte communities within the crowns of different-sized *Virola koschnyi* (Myristicaceae) emergent trees in a Costa Rican tropical wet forest. We tested the degree to which epiphyte species composition was associated with environmental conditions and resources (i.e. microhabitats) using multivariate analyses and a null model that compared the observed epiphyte assemblages amongst different-sized trees and crown zones with assemblages generated randomly. This study is the first to rigorously examine the degree of microhabitat specialization in epiphyte communities.

3. Microhabitat heterogeneity, epiphyte species richness and abundance increased with tree size. The largest trees had the highest microhabitat and epiphyte diversity and a unique inner crown microhabitat with canopy humus. The few epiphytes found on small trees were mostly bark ferns. Large trees had different epiphyte communities in different parts of the crown; the inner crown contained species not abundant in any other microhabitat (i.e. aroids, cyclanths and humus ferns), and the outer crown contained bark ferns and atmospheric bromeliads.

4. Variation in species composition amongst tree size classes was significantly related to the mean daily maximum vapour pressure deficit and tree diameter, while variation within large tree crowns was significantly related to canopy humus cover.

5. Microhabitat specialization of epiphyte species increased with tree size with 6% of species significantly associated with small trees and 57% significantly associated with large trees. Of the species present in large tree crowns, 23% were specialized to the unique inner crown microhabitat.

6. Synthesis. The increase in microhabitat heterogeneity within tree crowns as trees grow contributes to changes in epiphyte community structure, which supports decades-old hypotheses of the importance of microhabitat diversity and specialization in structuring tropical epiphyte communities.

Key-words: Costa Rica, determinants of plant community diversity and structure, La Selva Biological Research Station, microhabitat heterogeneity, microhabitat specialization, niche differentiation, randomization tests, *Virola koschnyi*

Introduction

The positive relationship between habitat heterogeneity and species diversity is amongst the best documented patterns in community ecology (Hutchinson 1959; Tews *et al.* 2004; Stein, Gerstner & Krefl 2014). Greater habitat heterogeneity

is associated with greater species diversity for many taxa including fishes (Messmer *et al.* 2011), mammals (Tews *et al.* 2004), birds (Kissling, Field & Böhning-Gaese 2008), insects (Kerr, Southwood & Cihlar 2001) and tropical woody plants (Clark, Clark & Read 1998a; Harms *et al.* 2001). Habitat heterogeneity may maintain species diversity if species show habitat specialization (e.g., Clark, Clark & Read 1998a; Harms *et al.* 2001; Kerr, Southwood & Cihlar 2001; Messmer

*Correspondence author. E-mail: cwoods@colgate.edu

et al. 2011). Most studies assume habitat specialization through correlative analyses between habitat and species distributions, but manipulative experiments or rigorous analytical techniques such as null models can better determine the relative contribution of habitat specialization to species diversity (Harms *et al.* 2001; DeWalt *et al.* 2006; Messmer *et al.* 2011).

One system in which habitat heterogeneity may contribute to species diversity is the tropical rain forest canopy. The rain forest canopy is host to a large proportion of the tropical diversity in wet tropical forests (Gentry & Dodson 1987) including up to 35% of the vascular floral diversity in vascular epiphytes (Nieder, Prosperí & Michaloud 2001). Vascular epiphytes are non-parasitic arboreal plants, most of which spend their entire life cycle within trees ('holoepiphytes' *sensu* Benzing 1990). There is a well-established, positive relationship between epiphyte diversity and tree size (e.g., Zotz & Vollrath 2003). Although an increase in epiphyte diversity with tree size may be a result of the increase in time for colonization and habitat area as trees become older and larger, changes in epiphyte diversity may also be influenced by the change in within-crown microhabitat heterogeneity that occurs with increasing tree size. Microhabitats in tropical canopies are formed by differences in environmental conditions (relative humidity, vapour pressure deficit; Cardelús & Chazdon 2005; de la Rosa-Manzano *et al.* 2014), structure (branch size, tree size; Johansson 1974; Hietz & Hietz-Siefert 1995) and resources (atmospheric deposition and gradients in light; Benzing 1990; Clark *et al.* 1998b; Cardelús *et al.* 2009). Another important resource for epiphytes that can vary with tree size is canopy humus, defined as the organic substrate that forms from the decomposition of plant material *in situ* and into which some epiphytes establish (Benzing 1990). Small trees are young, have a small area on which to establish and have little variation in environmental conditions, structures and resources in their crowns. In contrast, large trees are older with a larger habitat area and have steep environmental, structural and resource gradients both vertically (lower to upper canopy) and horizontally (trunk to outer crown; Johansson 1974; Hietz & Hietz-Siefert 1995; Chilpa-Galván *et al.* 2013). The change in microhabitat gradients with tree size may influence the distribution of epiphytic vascular plants, particularly if different microhabitats favour different sets of species.

Observations and hypotheses about epiphyte associations with particular microhabitats have a long history. The first observations that epiphytes were non-randomly distributed within tree crowns were made by Schimper (1888), in which he recognized distinct epiphyte communities based on microhabitat exposure, and Pittendrigh (1948), who qualitatively categorized epiphytic bromeliad species into sun and shade plants. Gradients in light, water availability and substrate features have been hypothesized to explain epiphyte distributions within tropical tree canopies for more than 120 years (e.g., Schimper 1888; Benzing, Seeman & Renfrow 1978; ter Steege & Cornelissen 1989; Hietz & Briones 1998; Reyes-García, Mejía-Change & Griffiths 2012), but no study to date has directly measured the association of epiphyte species with

microhabitats within tree crowns at the community scale. The epiphyte microhabitat association work carried out so far has been correlative and examined only in large trees (Johansson 1974; Freiberg 1996; Hietz & Briones 1998; Cardelús 2007) or has focused only on an individual component of the microhabitat (Hietz & Briones 1998; Cardelús 2007; Reyes-García, Mejía-Change & Griffiths 2012). To more fully understand whether habitat heterogeneity influences epiphyte diversity, many aspects of the microhabitat should be measured for multiple species. Different epiphyte species may be influenced by different habitat features, or habitat features may combine to create a gradient in some other factor.

In this study, we examined the extent to which environmental conditions, structural features and resources within different-sized tree crowns influence epiphyte communities in one host tree species, *Virola koschnyi* Warb. (Myristicaceae). We tested the hypotheses that microhabitat heterogeneity increases as trees increase in size (Fig. S1 in Supporting Information) and that this greater heterogeneity is important for structuring vascular epiphyte communities. Specifically, we asked the following questions: (i) How do microhabitats and epiphyte species richness, abundance and composition change with tree size? (ii) Are changes in epiphyte community composition with tree size associated with changes in microhabitats? and (iii) To what degree do species show habitat specialization amongst tree sizes or microhabitats within tree crowns? We would infer that microhabitat heterogeneity contributes to vascular epiphyte community structure from two findings: epiphyte species composition is explained by microhabitat factors as trees increase in size, and epiphyte species show significant associations with particular microhabitats. To our knowledge, this is the first study to measure multiple aspects of epiphyte microhabitats (i.e. environmental conditions, structures and resources) at the community scale and directly test the century-old hypothesis that microhabitat heterogeneity within tree crowns influences vascular epiphyte community structure.

Materials and methods

STUDY AREA

This study was conducted at La Selva Biological Research Station (10°25'52" N, 84°00'12" W, 40 m a.s.l.) in northeastern Costa Rica. The 1600 ha of the La Selva forest are characterized as tropical wet forest (Holdridge 1967), 55% of which is primary forest with an average density of trees ≥ 10 cm diameter at breast height (dbh) of 446.0 individuals ha⁻¹ (McDade *et al.* 1994). La Selva receives approximately 4000 mm of annual precipitation, predominantly during the wet season, May–January, with an average monthly precipitation of 382 mm. The drier season, February–April, receives an average monthly precipitation of 172 mm. Average monthly temperature is 25.8 ± 0.2 °C and varies little throughout the year (McDade *et al.* 1994).

We examined microhabitat associations of vascular epiphytes within the crowns of 61 individual *V. koschnyi* trees (Myristicaceae) ranging in dbh from 2.5 to 103.3 cm. *Virola koschnyi* are emergent, evergreen trees with abundant and diverse vascular epiphyte

communities along the branches but few along the trunk. Our study, therefore, focused on the crown alone. We chose *V. koschnyi* because the myristaceaceous branching pattern (branches radiate perpendicular to the bole) made it possible to survey the epiphyte community and measure microhabitat features within the different parts of the canopy (inner crown to outer crown). We chose five large trees (>70 cm dbh) from the 17 available *V. koschnyi* trees in the TREES data base (D. B. Clark and D. A. Clark, pers. commun.) that were healthy, were safe to climb and were located within old-growth forest. Our remaining trees (all sizes) were also located within old-growth forest and were >5 m from the main trails. Our study area covered approximately 1300 ha of the La Selva forest, in which the density of *V. koschnyi* trees ≥ 10 cm dbh is 4.1 individuals ha^{-1} and the density of trees ≥ 60 cm dbh is 0.4 individuals ha^{-1} (D. B. Clark and D. A. Clark, unpubl. data).

We chose one species of tree to control for variation in host characteristics, such as bark physiognomy, humus chemistry, foliar leaching and life history (evergreen vs. deciduous, Cardelús 2007; Cardelús *et al.* 2009). Despite only examining one tree species, the findings of our study are likely applicable for other tree species as epiphytes show little host-species specificity (Laube & Zotz 2006). We included holoeiphytes (i.e. non-parasitic arboreal plants that spend their entire life cycle in the tree canopy) and some primary hemieiphytes (i.e. those that begin their life epiphytically and eventually connect to terrestrial soil) in our survey. There were no secondary hemieiphytes (i.e. those that begin rooted in terrestrial soil and lose their connection, Benzing 1990; also referred to as 'nomadic vines' in Moffett 2000).

EPIPHYTE SURVEYS

We surveyed epiphyte communities using both within-tree and ground-based surveys. All trees >70 cm dbh and several trees ≤ 70 cm dbh whose crowns were not easily visible from the ground and were safe to access were climbed using single-rope climbing techniques (Perry 1978), and epiphytes were surveyed on 5–6 branches every 1 m from the trunk to the branch tips. Only the lower 5–6 branches were surveyed, as it was logistically impossible to reach and properly survey higher branches. Within the crowns of the largest trees (i.e. >70 cm dbh), we designated three crown zones based on distance from the trunk: inner (0–2 m), mid (2–5 m) and outer (>5 m) crown, in accordance with Johansson (1974; Fig. S1). Ground-based epiphyte surveys were conducted for the remaining trees ≤ 70 cm dbh as the branches of these trees were too small to climb, the branches were visible from the ground (i.e. branches were 4–20 m from the ground), and ground-based surveys have been shown to capture >90% of large epiphyte occurrences (Burns 2007). Individuals <10 cm leaf length whose identity could not be determined were not included in analyses examining changes in epiphyte communities with tree diameter. For instance, bark ferns and *Peperomia* spp. were easy to identify given their rhizomatous growth and were included in all analyses. However, small orchids, small aroids and small bromeliads were not easily identifiable to species and, therefore, were included only in abundance analyses. Given the low abundance of small orchids and small aroids in *V. koschnyi* trees (<5 individuals), they would not have been included in our compositional analyses (see below), and therefore, their exclusion did not influence those results. The high abundance of small bromeliads, however, could result in an underestimate of species richness in all trees and an overestimate in the similarity in species composition amongst small trees if the small bromeliads represented multiple species. Furthermore, slow-growing species may not be included in small trees if they

were too small to detect when young, which could underestimate similarity in species composition between small and large trees.

We classified each epiphyte species into groups defined *a priori* by taxon: aroids, bromeliads, cacti, ferns and orchids. We further partitioned bromeliads based on nutrient uptake mechanisms into tank bromeliads (impounding) and atmospheric bromeliads (non-impounding) as was done by Benzing, Seeman & Renfrow (1978), and we partitioned ferns based on rooting medium into humus ferns (always root in humus) and bark ferns (independent of rooting medium) following *Scheme V* in Benzing (1990).

MICROHABITAT MEASUREMENTS

To determine the diversity of microhabitats within tree crowns, we measured microhabitat variables hypothesized to be related to epiphyte species distributions. The microhabitat variables measured were temperature (*T*), relative humidity (RH), vapour pressure deficit (VPD), % canopy humus cover and crown illumination or % canopy openness. Temperature and RH were measured in the inner crowns of 3–5 individuals in each tree size class (see Statistical analysis below for tree size classes determined from tree diameters) and in the mid- and outer crowns of the five trees in the largest tree size class using LogTag dataloggers (MicroDAQ, Contoocook, NH, USA) that recorded *T* and RH every 6 min for at least 8 weeks during the wet season (July–September) in 2011 from which we calculated VPD. We calculated the average of the maximum daily VPD (mean daily max VPD) across the 8-week period. Temperature and RH could not be measured in the mid- and outer crowns of smaller tree size classes due to logistical constraints.

We examined the amount of light reaching the entire crowns of 5–20 *V. koschnyi* trees from each size class using the crown illumination index (CII), an ordinal scale used to assess the amount of light exposure of each tree crown. CII ranges from a value of 1, which is a crown with no direct light reaching it from the side or the top and an average canopy openness of 4%, to a value of 5, which is a completely exposed crown with light reaching it from all sides and the top and an average canopy openness of >90% (Clark & Clark 1992; Keeling & Phillips 2007). Within the largest trees, we measured branch diameters in the inner and mid-crown and estimated diameter for the outer crown; we measured % canopy openness in the inner crown using a densitometer (Forestry Suppliers Inc., Jackson, MS, USA) and estimated the cover in the mid- and outer crown. Our % canopy openness values were similar to those measured by Johansson (1974) for the inner, mid- and outer crown. We estimated the percentage of the branch covered by canopy humus in the inner and mid-crowns of each branch in each tree by placing a 1-m² quadrat over the branch that was 0.5 × 2 m². The quadrats were divided into 100 squares that were 10 × 10 cm². We counted the number of squares in which canopy humus was present to get the proportion of branch that was covered in canopy humus (Krebs 1999). The outer crowns of the largest trees had no humus and were given a per cent humus cover of zero.

STATISTICAL ANALYSIS

Microhabitats and community structure

To examine how epiphyte species richness and epiphyte abundance changed with tree size, we used regression. We predicted that epiphyte species richness and abundance would increase with tree diameter following either a linear, loglinear (i.e. exponential) or polynomial (i.e. quadratic) model based on previous studies (Zotz & Vollrath 2003). We, therefore, determined the best fit model from a linear,

exponential or quadratic model as the one with the lowest second-order Akaike information criterion that controls for small sample size (AICc, Burnham & Anderson 2002). To check if the assumptions of the model were met, we examined $Q-Q$ plots, the distribution of residuals and plots of residuals vs. predicted values. All of the models tested met the assumptions. We also used regression to examine how microhabitat variables changed with tree size, including the mean daily max VPD and CII, and how microhabitat variables and branch diameter changed with distance from the trunk in the largest trees; microhabitat variables included mean daily max VPD, % canopy humus cover and % canopy openness. We followed the same procedures as above for model selection to determine whether a linear, log-linear or quadratic model fit the microhabitat variables. For CII, we also examined a logarithmic model as we hypothesized that CII would increase until the trees emerged from the canopy and then would level off. Some of these relationships were found previously in tree canopies (e.g., Johansson 1974; Hietz & Hietz-Siefert 1995).

To examine whether the changes in epiphyte community composition were influenced by the changes in microhabitats that accompany tree size, we used canonical correspondence analysis (CCA), a multivariate ordination technique that constrains the ordination by the environmental variables chosen. We confined our analysis to epiphyte species with a total of >5 individuals in the entire survey of all trees ($n = 68$ for tree size analysis and $n = 60$ for crown zone analysis). Microhabitat variables that were examined amongst tree sizes were CII and mean max daily VPD, and microhabitat variables that were examined amongst crown zones within the largest trees were mean daily max VPD, branch diameter, % canopy humus cover and % canopy openness. Amongst crown zones, % canopy humus cover, branch diameter and % canopy openness had high multicollinearity, and therefore, branch diameter and % canopy openness were not included in the CCA. We examined which microhabitat factors explained a significant amount of variation in species compositional differences amongst tree size classes and amongst crown zones using stepwise forward-selection procedures and Monte Carlo permutation tests (1000 permutations). We used the 'cca' function and the 'ordistep' function in the vegan package of R version 3.0.1 for CCA and stepwise forward-selection, respectively (R Development Core Team 2009; Oksanen *et al.* 2010). We used a Mantel test to examine if epiphyte species composition amongst tree size classes was related to geographic location using a Bray–Curtis dissimilarity matrix of species composition and a Euclidean matrix of geographic location.

We used minimum convex hulls in the CCA to denote variance in species composition amongst trees of similar size to better visualize shifts in species composition with tree diameter and amongst crown zones within large trees. We defined tree size classes based on shifts in species composition using multivariate regression trees (MRT). MRT is a multivariate discrimination technique that forms clusters of sites based on environmental variables that minimize the dissimilarity of groups within clusters (De'Ath 2002). We used epiphyte abundance and species richness to define our tree clusters using a Bray–Curtis dissimilarity matrix. The MRT analyses created four tree size classes: 15 cm = 2.5–15 cm dbh; 30 cm = 15.1–30 cm dbh; 70 cm = 30.1–70 cm dbh; and >70 cm dbh (Fig. S2). Results amongst size classes for the CCA and microhabitat associations did not vary when size classes were changed.

Test of microhabitat associations

We examined the association of epiphyte species to each of the four tree size classes as well as the three crown zones within the largest

trees (six microhabitats in total: 15 cm, 30 cm, 70 cm, >70 cm inner crown, >70 cm mid-crown and >70 cm outer crown) using conservative randomization tests. These randomization tests are null models that take into account the non-random and often clumped dispersion of plants and have previously been used to examine habitat associations of liana species (DeWalt *et al.* 2006). We tested habitat associations for epiphyte species with >14 individuals in the entire survey of all trees ($n = 33$) in accordance with DeWalt *et al.* (2006). The model compared the observed relative proportion of each species in each microhabitat to the null expected relative proportion generated by 1000 iterations of shuffling the six microhabitats. The observed relative proportion of a species in a particular microhabitat was calculated for each tree size category or crown zone by computing the average proportion across individual tree size classes or crown zones. The relative proportion in a particular tree size class or crown zone was calculated as the proportion of epiphytes composed by that species in a particular tree or crown position. A positive or negative association with a particular microhabitat resulted when the observed proportion was greater or less than the expected proportion for more than 97.5% of the randomizations, respectively (two-tailed test with $\alpha = 0.05$).

Results

Within 61 *V. koschnyi* trees, we found 6250 epiphyte individuals representing 97 species in 44 genera and 15 families (Table S1). Within this one tree species, we found 30% of all epiphyte species at La Selva (McDade *et al.* 1994) and observed a maximum of 65 species in a single tree (minimum of 0 and mean of 7.8 species \pm 1.85 SE). Most individuals surveyed were members of the Bromeliaceae (64%) with 7 genera and 18 species and the Orchidaceae (7%) with 12 genera and 30 species (Table S1). Bromeliaceae composed a quarter to a half of all epiphytes on trees >15 cm dbh (Table S1).

The number of species and individuals increased significantly with tree size (Fig. 1). The increase in species richness with tree diameter was best described by a loglinear model [Fig. 1a; AICc = 14.83, $R^2 = 0.77$; $\log(\text{species richness}) = 0.02 (\pm 0.001, 1 \text{ SE}) \text{ dbh} + 0.001 (\pm 0.05)$] relative to a linear model (AICc = 394.22, $R^2 = 0.83$) or a polynomial model (AICc = 374.89, $R^2 = 0.88$). Similarly, the increase in epiphyte abundance with tree diameter was best described by a loglinear model [Fig. 1b; AICc = 89.43, $R^2 = 0.73$; $\log(\text{abundance}) = 0.03 (\pm 0.002) \text{ dbh} + 0.2 (\pm 0.09)$] relative to a linear model (AICc = 769.35, $R^2 = 0.77$) or a polynomial model (AICc = 689.13, $R^2 = 0.94$). With increasing tree size, more epiphyte groups were found, and the largest trees hosted all seven groups (Fig. 2; Table S1). The few epiphytes that were found on small trees were mostly bark ferns (Fig. 2).

Large trees had different epiphyte communities in different parts of the crown. The epiphyte community in the outer crown zone of large trees was compositionally more similar to smaller tree crowns that were kilometres away than to the inner crown of large trees that were metres away (Fig. 2). In the inner crown zone, the fern community changed from dominance by bark ferns on small trees to dominance by humus ferns in large trees. The inner crown zone of the largest trees had species and epiphyte groups that were not

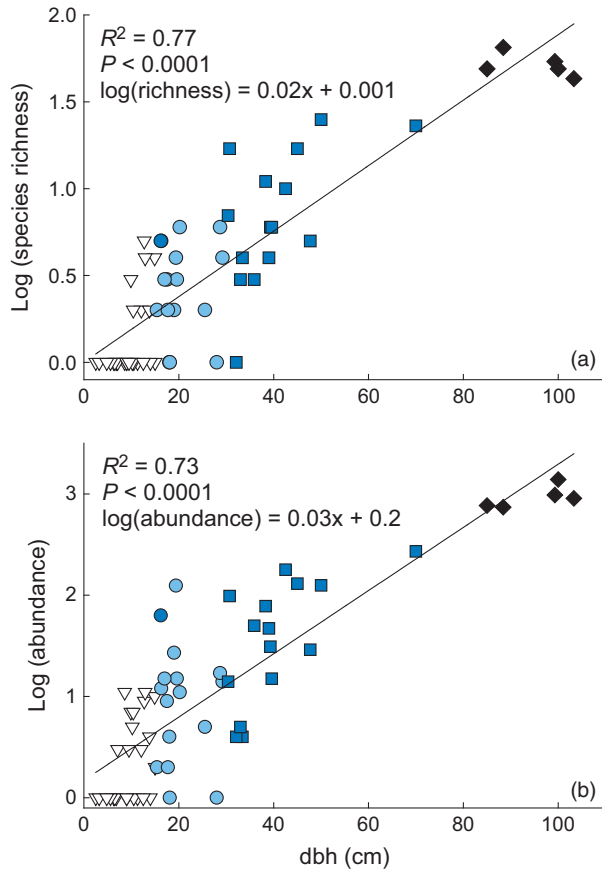
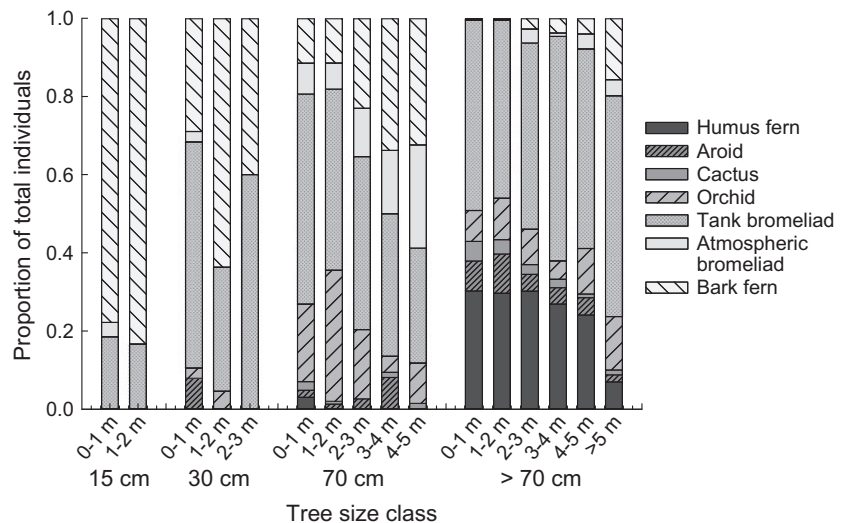


Fig. 1. Relationship between epiphyte species richness (a) and epiphyte abundance (b) with tree diameter (dbh) for 61 *Virola koschnyi* trees from lowland wet rain forest at La Selva Biological Research Station in Costa Rica. Different symbols denote tree size classes: 15 cm = 2.5–15 cm, downward-facing triangles; 30 cm = 15.1–30 cm, circles; 70 cm = 30.1–70 cm, squares; and >70 cm, diamonds.

abundant in any other tree size class or crown zone (i.e. aroids, cyclanths and humus ferns), all of which were never found without canopy humus around their roots (Fig. 2; Table S1).

Fig. 2. Epiphyte composition (expressed as the proportion of different epiphyte groups) found at different distances from the tree trunk in the crowns of different size classes of 61 *Virola koschnyi* trees from lowland wet rain forest at La Selva Biological Research Station in Costa Rica.



Microhabitats changed with tree size and distance from the trunk in the largest trees and influenced species distributions. Microhabitat factors varied in their relationship with tree size. Mean daily max VPD was best described by a humped distribution (Fig. 3a), and CII was best described by a linear relationship (Fig. 3b), as no other model for either factor met the normality assumptions. Only trees >70 cm dbh had canopy humus. All within-crown habitat factors examined with distance from the trunk were best described by a linear relationship. Per cent canopy humus cover and branch diameter significantly decreased, and mean daily max VPD and % canopy openness significantly increased with distance from the trunk (Fig. 4). The CCA showed that measured microhabitat features explained 57% of the variation in species composition amongst tree size classes. Variation in species composition amongst tree size classes was significantly related to mean daily max VPD ($F_{1,23} = 4.6$, $P = 0.01$) and dbh ($F_{1,23} = 3.6$, $P = 0.04$; Fig. 5a). Within large tree crown zones, microhabitat factors explained 41% of the variation in epiphyte species composition. The first CCA axis was negatively related to % canopy humus cover and positively related to mean daily max VPD (Fig. 5b). However, only humus cover was significantly associated with species composition ($F_{1,13} = 3.6$, $P = 0.01$).

Epiphyte species composition was not related to the geographic location of the *V. koschnyi* trees at La Selva (Mantel test, $r = -0.006$, $P = 0.50$).

MICROHABITAT ASSOCIATIONS

Twenty-five of the 33 species (76%) exhibited a significant association with one of the six microhabitats, defined by tree size and crown zone (Table 1). Species within epiphyte groups showed similar significant microhabitat associations related to the distribution of microhabitats within the crowns of the largest tree size class. For example, of the eight species of humus ferns and aroids, seven showed a positive association with the inner or mid-crown zone of large trees where

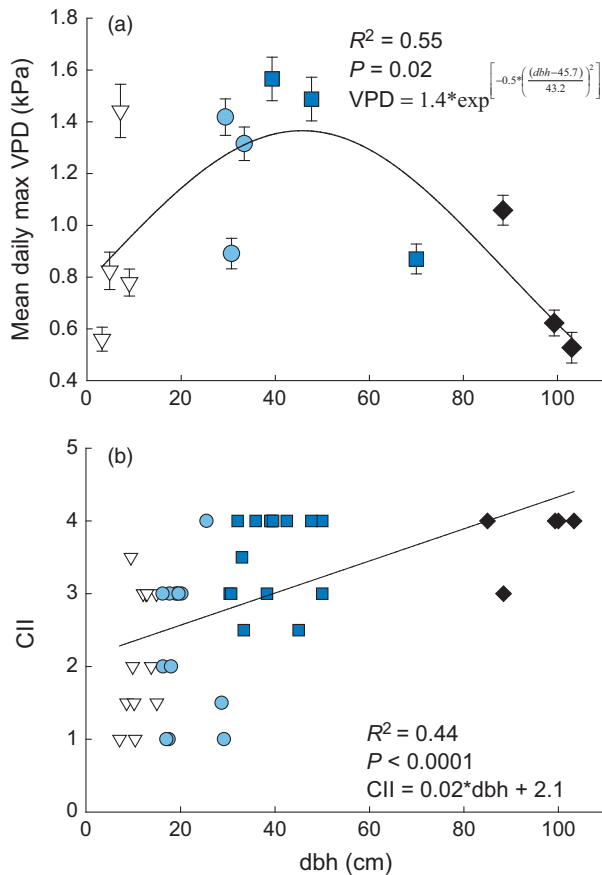


Fig. 3. Relationship between inner crown mean daily max VPD (kPa) (a) and canopy illumination index (CII; b) with tree diameter (dbh) for (a) 13 *Virola koschnyi* trees and (b) 61 *V. koschnyi* trees ranging in dbh from 2.5 to 103.3 cm from lowland wet rain forest at La Selva Biological Research Station in Costa Rica. Different symbols denote tree size classes: 15 cm = 2.5–15 cm, downward-facing triangles; 30 cm = 15.1–30 cm, circles; 70 cm = 30.1–70 cm, squares; and >70 cm, diamonds.

canopy humus was available. Six of the 10 tank bromeliad species showed negative associations with small tree size classes, and two species showed a positive microhabitat association with the largest tree size class. Only one bark fern species and a species of *Peperomia* showed positive associations with small trees (Table 1).

Discussion

As *V. koschnyi* trees increased in size, vascular epiphyte diversity and abundance also increased. This well-documented relationship has been attributed to an increase in habitat area and time for colonization as trees increase in size and age (Zotz & Vollrath 2003; Woods & DeWalt 2013), but our study shows that it also arises because of greater heterogeneity in microhabitats that develops within tree crowns as they grow larger. In our study, 57% of the variation in species composition amongst tree size classes and 41% of the variation in species composition amongst crown zones within large trees were explained by microhabitat variables, and 76% of epiphyte species showed an association with a particular tree size or microhabitat within *V. koschnyi* tree crowns. The largest trees had the greatest microhabitat heterogeneity and the highest percentage of epiphyte species with a significant microhabitat association. Six plant families, 23 genera and 56 species were unique to the largest tree size class (Table S1); 32% of species unique to large trees were significantly associated with the inner crown (Table 1). If greater area and more time solely influenced the change in epiphyte community structure with tree size, the species distributions within tree crowns would be random and any microhabitat association would be due to chance. Our study found that greater area and more time, as indirectly measured with tree size, contribute to the positive relationship between species richness and abundance with

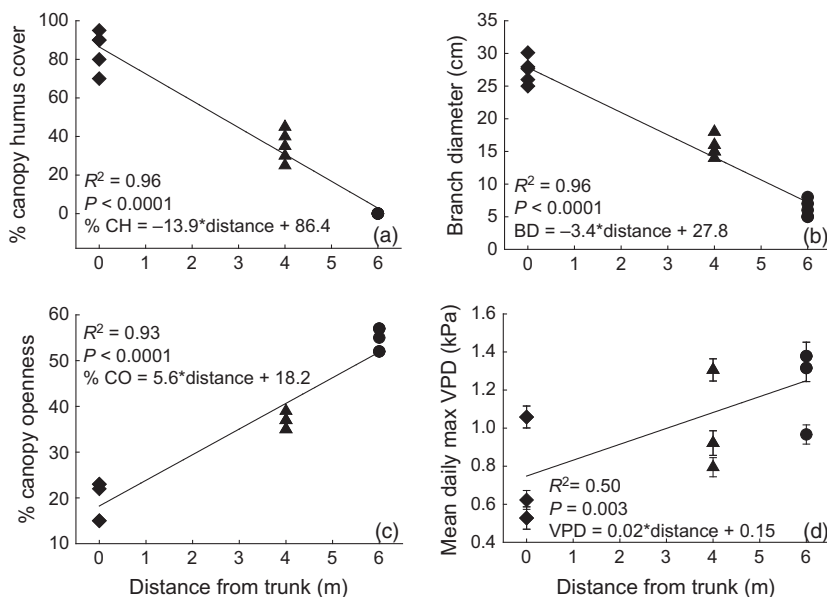


Fig. 4. Relationship between distance from the trunk and average % canopy humus cover (CH; a), branch diameter (BD; b), % canopy openness (CO; c) and mean daily max VPD (VPD; d) within five *Virola koschnyi* trees > 70 cm dbh from lowland wet rain forest at La Selva Biological Research Station in Costa Rica. Different symbols denote crown zones: diamonds = inner crown (0–2 m); upward facing triangles = mid-crown (2–5 m); and circle = outer crown (> 5 m). Some symbols are not visible because they are on top of each other.

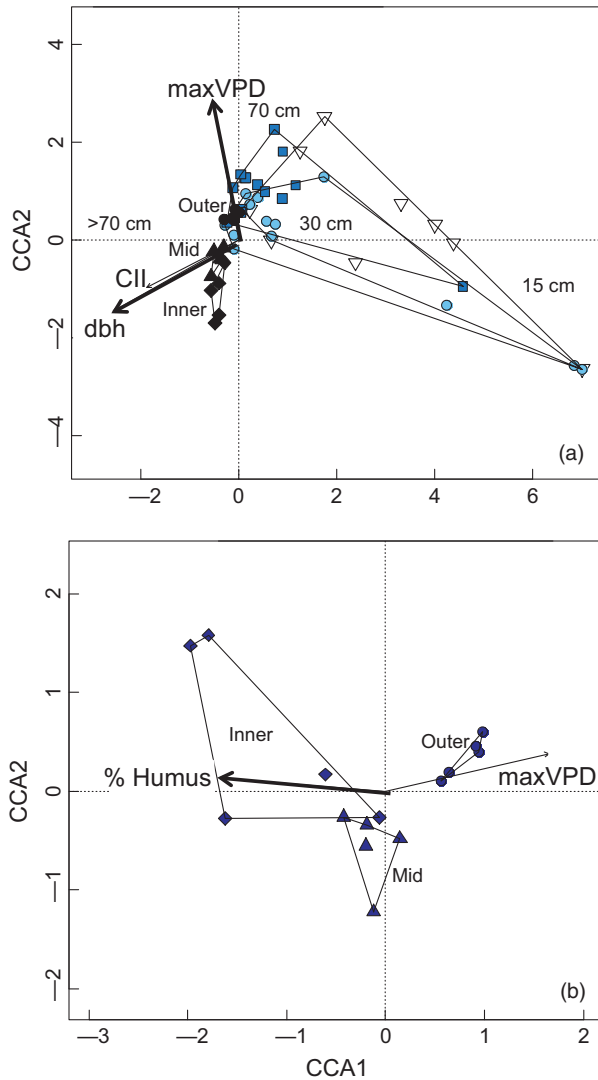


Fig. 5. Canonical correspondence analysis (CCA) ordination of epiphyte community composition within the crowns of 5–25 *Virola koschnyi* trees from each of four size classes from lowland wet rain forest at La Selva Biological Research Station in Costa Rica for all trees and with the largest trees separated into crown zones (a) and for crown zones in the largest tree class (b). Different symbols denote tree size classes: 15 cm: 2.5–15 cm, downward-facing triangles; 30 cm: 15.1–30 cm, circles; 70 cm: 30.1–70 cm, squares; and >70 cm, diamonds. For (b), trees >70 cm dbh were separated into crown zones: diamonds = inner crown (0–2 m); upward facing triangles = mid-crown (2–5 m); and circle with a cross = outer crown (>5 m). Environmental variables that explain a significant proportion of variation in species composition are shown as black, heavy arrows, and non-significant environmental variables are shown as grey arrows. max VPD is the mean daily max VPD, and % humus is the % canopy humus cover. Lines represent the minimum convex hulls for each tree size and crown zone.

tree size, but they do not explain the non-random distribution of epiphytes within tree crowns or the number of microhabitat associations within the largest tree crowns. Thus, increasing tree size produces a greater diversity of microhabitats in the crown, which contributes to greater epiphyte diversity through microhabitat specialization.

MICROHABITAT HETEROGENEITY AND SPECIES DISTRIBUTIONS

Tree crown microhabitats in the smaller size classes were homogeneous with little variation from the inner to the outer crown zone – they had no canopy humus and little variation in microhabitat structure and resources. The few epiphyte species and groups that were found in small trees included bark ferns (i.e. *Microgramma* sp.) and a few *Peperomia* spp. These groups were also found in the outer crowns of large trees, which may be due to their ability to live on bare bark as well as to tolerate variable light levels, such as low light in small understorey trees as well as high light in the outer crown of large trees.

Larger trees had greater microhabitat heterogeneity within their crowns with substantial changes from the inner to the outer crown in branch diameter, % canopy humus cover, % canopy openness and mean daily max VPD. The inner crown zone changed the most with increasing tree size. Larger trees had greater amounts of canopy humus and had unique environmental conditions in this zone relative to the rest of the crown. In the inner crown zones of the largest trees, VPD was lower, RH was higher, and light was lower than in the outer and mid-crown zones. The lower light levels of inner crown zones in larger trees were likely due to self-shading by the tree, which is shown by canopy openness in the inner crown of large trees being 20% despite the CII value of large trees (i.e. 4) being equivalent to an average of 37% of visible sky (Keeling & Phillips 2007). Lower mean daily max VPD and higher RH in inner crown zones of large trees may result from large mats of canopy humus that hold water for longer periods of time than bare bark, higher tree leaf transpiration in the inner crown and trapped water vapour from the canopy boundary layer. The inner crown zone of these large trees had a unique epiphyte community made up of humus ferns, aroids and cyclanths that were significantly associated only with microhabitats where canopy humus was present.

All microhabitat factors measured explained the non-random distribution of epiphyte species amongst crown zones within large trees. Our results are consistent with other studies that found epiphyte distributions within large trees to be correlated with the size of branches (Johansson 1974; ter Steege & Cornelissen 1989; Hietz & Hietz-Siefert 1995), the exposure of the crown (Pittendrigh 1948; Johansson 1974; ter Steege & Cornelissen 1989), microclimate (Cardelús & Chazdon 2005) and canopy humus (Freiberg 1996). Given the high multicollinearity amongst measured characteristics of the microhabitat in our study, it is likely that these features combine to create a gradient in some other unmeasured factor, such as drought stress, as has been proposed previously (e.g., Benzing, Seeman & Renfrow 1978; ter Steege & Cornelissen 1989; Hietz & Briones 1998; Reyes-Garcia, Mejia-Change & Griffiths 2012). Species that had significant associations with the inner crown zone of large trees, such as many humus ferns, are less adapted to drought than those found in the outer crown, such as bark ferns and atmospheric bromeliads (Pittendrigh 1948; Benzing, Seeman & Renfrow 1978;

Table 1. Results from the null models (randomization tests) showing significant positive (+), negative (–) or absent (no symbol) microhabitat associations of abundant epiphyte species from different epiphyte groups to different tree size classes or canopy zones within the largest tree size class of *Virola koschnyi* at La Selva Biological Research Station in Costa Rica. A positive or negative association with a particular microhabitat resulted when the observed proportion was greater or less than the expected proportion for more than 97.5% of the randomizations. No association means that the species' distribution amongst microhabitats is not different from random. Tree size classes are defined as: 15 cm = 2.5–15 cm; 30 cm = 15.1–30 cm; 70 cm = 30.1–70 cm; and >70 cm. Crown zones are defined as: inner crown (0–2 m from trunk); mid-crown (2–5 m); and outer crown (>5 m). Abundance of each species is included in parentheses beside species' names

Epiphyte group/Family	Species	>70 cm inner	>70 cm mid	>70 cm outer	70 cm	30 cm	15 cm
Aroids	<i>Anthurium ramonense</i> (32)	+				–	–
	<i>Anthurium upalaense</i> (75)				–		–
	<i>Philodendron wendlandii</i> (15)	+	+				
	<i>Stenospermation angustifolium</i> (79)		+				
Atmospheric bromeliads	<i>Tillandsia bulbosa</i> (73)			+	–	–	
	<i>Tillandsia festuroides</i> (90)						
Bark ferns	<i>Microgramma lycopodioides</i> (118)						
	<i>Microgramma percussa</i> (56)						
	<i>Microgramma reptans</i> (227)	–	–				+
Cacti	<i>Rhipsalis baccifera</i> (37)					–	–
	<i>Epiphyllum hookeri</i> (28)						
Cyclanthaceae	<i>Chorigyne pendula</i> (63)		+				
	<i>Sphaeradenia acutitepala</i> (12)	+					
Gesneriaceae	<i>Codonanthe</i> sp. (14)			+			
Orchids	<i>Nidema boothii</i> (224)			+			
	<i>Elleanthus cynarocephalus</i> (14)		+				
	<i>Prosthechea abbreviata</i> (69)						
	<i>Pleurothallis</i> sp. 1 (16)	+					
Piperaceae	<i>Peperomia rotundifolia</i> (172)					+	+
Humus ferns	<i>Elaphoglossum herminieri</i> (212)	+			–	–	–
	<i>Elaphoglossum latifolium</i> (220)	+	+		–	–	
	<i>Phlebodium pseudoaureum</i> (42)	+					
	<i>Vittaria lineata</i> (120)	+	+				–
Tank bromeliads	<i>Aechmea nudicaulis</i> (392)					–	–
	<i>Guzmania lingulata</i> (382)						
	<i>Guzmania monostachya</i> (386)						–
	<i>Guzmania desautelsii</i> (35)						
	<i>Tillandsia anceps</i> (320)				–	–	
	<i>Tillandsia monadelpha</i> (175)	+				–	–
	<i>Tillandsia venusta</i> (12)						
	<i>Vriesea vittata</i> (17)			+			
	<i>Werauhia gladioliflora</i> (168)						–
	<i>Werauhia kupperiana</i> (123)					–	–

Benzing 1990; Watkins *et al.* 2007). Canopy humus buffers epiphytic plants from experiencing extremely dry conditions in the canopy as it absorbs rainwater and has low rates of drainage and evaporation (Veneklaas *et al.* 1990; Freiberg 1996). Thus, differences in strategies to avoid or tolerate drought may explain the non-random distribution of epiphyte species within tree crowns.

The distributions of species that lack a relationship to any measured microhabitat characteristic may be due to dispersal limitation (Hubbell 2001), an unmeasured microhabitat variable, density- or frequency-dependent mortality imposed by natural enemies (Connell 1971) or a wide tolerance to different microhabitats (i.e. are generalists). In our study, tank bromeliads showed little distributional relationship to microhabitat gradients within tree crowns and therefore may be microhabitat generalists. Several tank bromeliad species, including *Aechmea nudicaulis* and *Werauhia kupperiana*, showed significant negative associations with small trees,

which may be due to their large size as adults and the inability of small branches to hold them. However, only two species (*Tillandsia monadelpha* and *Vriesea vittata*) showed any significant association with a particular crown zone within the largest trees. Tank bromeliads uptake water and nutrients from phytotelmata through leaf trichomes and use their roots solely for anchorage to their host tree (Benzing 1990). The ability of tank bromeliads to store water and access nutrients from a variety of sources reduces the likelihood that they are tied to particular substrate characteristics as hypothesized by Pittendrigh (1948), like other epiphyte taxa are, and may explain their wide distribution.

THE IMPORTANCE OF LARGE TREES

Our results highlight the importance of microhabitat diversity and specialization in structuring some tropical plant communities. Although time and area for colonization contribute

substantially to the development of epiphyte communities, the greater diversity of microhabitats in older, larger trees also promotes epiphyte diversity. The lack of large trees and a well-developed humus layer in the inner crown early in succession likely explains the low diversity and absence of old-growth epiphyte species in secondary forests (Barthlott *et al.* 2001), even in old secondary forests >115 years of age (Woods & DeWalt 2013). Understanding the small-scale microhabitat requirements of species therefore enables a better understanding of large-scale distributions of tropical vascular epiphytes. Furthermore, our results provide additional evidence of the importance of large, old trees as 'keystone ecological structures' (Lindenmayer *et al.* 2014) and further support for trying to mitigate the global decline of large, old trees (Lindenmayer, Laurance & Franklin 2012).

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Data accessibility

Epiphyte families in each tree size class: uploaded as online Supporting information.

A list of epiphyte species found in all trees in our survey: uploaded as online Supporting information.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Hypothesized gradients in microhabitats among tree size classes and amongst crown zones within the largest trees.

Figure S2. Multivariate regression tree used to define tree size classes.

Table S1. List of epiphyte species, their family and group within 61 *Virola koschnyi* trees at La Selva Biological Research Station in Costa Rica.

Table S2. Number of epiphyte families, genera, species, functional groups and individuals of each epiphyte family in total and within each *Virola koschnyi* size class.