

A field experiment links forest structure and biodiversity: epiphytes enhance canopy invertebrates in Chilean forests

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Abstract. Epiphytes represent a conspicuous component of the canopy of old-growth forests, reaching biomass values of up to 44 t/ha. Forest epiphytes host a rich invertebrate community; however, the contribution of epiphytes to the richness and abundance of canopy invertebrates is still quite unknown, and has not been experimentally assessed in old-growth forests. We studied the contribution of epiphyte loads to invertebrate species composition and abundance in the crown of large old individual trees of *Eucryphia cordifolia* (Cunoniaceae) in old-growth forests from southern Chile. We accessed the canopy using arborist techniques and contrasted the invertebrate species richness and biomass inhabiting four large canopy trees (25–30 m high, 1.2 m DBH), two of them with intact epiphytes and two trees from which epiphytes were manually removed. For over a year (April, 2006 to May, 2007) we made monthly collections of invertebrates from each tree's crown using flight-interception and elector traps (the latter designed to capture walking invertebrates from trunks and limbs). Once every season we collected samples of epiphytes and their soil to quantify invertebrates using Berlese funnels. We found significantly greater invertebrate species richness and abundance in the control trees' crowns (with epiphytes) compared to the trees from which epiphytes were removed. Predators (such as spiders and centipedes) were disproportionately more abundant on trees with epiphytes, and an entire functional group (detritivores), associated with arboreal soils under the epiphytes, was absent in the trees from which epiphytes were removed. Invertebrate abundances were lower in winter and higher in summer for trees with epiphytes, while for trees without epiphytes invertebrate numbers fluctuated markedly, but with no seasonal pattern. We show that large old trees are an important structural component in forests, supporting additional structure represented by the epiphyte load, which, in turn, sustains a rich community of invertebrates with functional groups not otherwise present in the forest canopy. Our findings strongly recommend that forest managers retain large old trees with their epiphytes in order to sustain biodiversity and important ecosystem processes.

Key words: canopy ecology; epiphytes; *Eucryphia cordifolia*; functional groups; invertebrate species richness; old-growth forests.

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INTRODUCTION

Biodiversity is defined as the diversity of living forms at different scales, from genes to ecosystems (Groom et al. 2006). Noss (1990; following Franklin et al. 1981) developed a framework to characterize biodiversity based on three main axes: composition, structure and function, which interact with each other at different levels of system organization. Composition refers to the identity of biodiversity units, such as the diversity of genes or alleles within organisms, or the taxonomic composition of communities. Structure refers to how these units are physically arranged, creating physical structural complexity. For instance, the physical structures created by logs or snags in forests. Lastly, functions refer to the biological interactions and biogeochemical processes that take place within ecological systems, such as gene flow, trophic interactions, nitrogen fixation and nutrient cycling. Noss (1990) proposed that these axes are interconnected, for instance, species diversity (composition) is linked to habitat structure and ecosystem processes.

Structures that characterize many old-growth forest ecosystems are large old canopy trees. These trees provide supporting structure and resources to a large number of forest species, such as a vast community of epiphytes and cavity nesting birds (Franklin et al. 1981, McCune 1993, Nadkarni et al. 2004, Johansson et al. 2007, Díaz et al. 2010). Epiphytes represent about 10% of all known vascular plants (Benzing 1995) and from 25–50% of the forest plant species in tropical and temperate rainforests (Gentry and Dodson 1987, Nieder et al. 2001). The “epiphyte loads” of large trees (up to 44 t/ha) are composed of the living tissues of epiphytes plus a dense layer of organic detritus and litter (Hofstede et al. 1993, Ingram and Nadkarni 1993, Enloe et al. 2006). This detritus which accumulates on the tree’s crown has been called “arboreal soil” because it is structurally and functionally similar to the organic horizon on the forest floor (Enloe et al. 2006).

Additionally, epiphyte loads can support a rich community of invertebrates. Nadkarni and Longino (1990) recorded a similar abundance of

invertebrates in arboreal soil and on the forest floor, with the presence of decomposers, ants and mites. Ellwood and Foster (2004) found that a single mat of epiphytes in Borneo’s dipterocarp forest holds as much arthropod biomass, including decomposers and mites, as the foliage and branches of the host tree. Yanoviak et al. (2007) found a striking abundance of mites and ants in soil from epiphytes of Monte Verde forest, Costa Rica, while Cruz-Angón et al. (2009) found that in shaded coffee plantations in Mexico the diversity of invertebrates was higher on trees with epiphytes than on trees with no epiphytes. These studies indicate that the ecology of epiphyte assemblages is distinct from the ecology of tree crowns alone, with invertebrate functional groups reliant on epiphytes, such as earthworms and ground mites, which are quite different from those that dwell in the tree foliage (Stuntz et al. 2003, Winchester and Behan 2003, Ellwood and Foster 2004, Yanoviak et al. 2003, 2007). However, the vast majority of studies on canopy invertebrates have assessed their diversity at the whole tree scale (e.g., Clement et al. 2001, Kitching et al. 2002, Basset et al. 2003, Arias et al. 2008), thus obscuring epiphytes’ specific contribution to the species composition of canopy assemblages and their functions.

The South American temperate rainforest (SAF), which extends along the western side of the Andes between 35° and 55° S, are dominated by evergreen, broad-leaved tree species, which are densely covered by a rich epiphyte community of vascular and non-vascular plants (Armeseto et al. 1998, Clement et al. 2001, Pérez et al. 2005, Díaz et al. 2010). Large trees can hold loads of over 130 kg of dry epiphyte biomass (Díaz et al. 2010); sufficient epiphyte matter to support substantial invertebrate assemblages (Yanoviak et al. 2007). Here, we analyze the distinctive contribution of epiphyte loads to the composition and temporal variation of canopy invertebrate communities (Stuntz et al. 2003, Yanoviak et al. 2003, 2007). By a field experiment, we compared the invertebrate assemblages associated with whole emergent trees from which epiphytes were previously removed to paired control trees with intact epiphyte cover. The advantage of our field experiment over previous observational

studies is the ability to separate the specific effects of epiphytes on invertebrate species assemblages from the effect of the tree itself, providing strong inference concerning the role of epiphytes in supporting canopy biodiversity (Quinn and Dunham 1983).

We hypothesized that epiphytes contribute substantially to enriching canopy invertebrate assemblages and predicted that large trees with high epiphyte biomass would support greater abundance, species richness and functional groups of invertebrates than similar-sized trees from which epiphytes were experimentally removed. Finally, we discuss the functional link between the structure represented by old trees with epiphytes and the species composition of invertebrates, and the importance of conserving large old trees for the biodiversity of forest ecosystems.

METHODS

Study area

We worked in an extensive patch (>200 ha) of old-growth Valdivian temperate rainforest in the vicinity of Guabún, Chiloé Island, Chile (41°47' S; 54°00' W; Fig. 1). The forest patch sits in a mosaic of about 1000 ha of continuous forest, with different degrees of human impact and successional ages. The forest is dominated by broad-leaved, evergreen trees and has a multi-layered canopy with the frequent presence of large, canopy emergent trees of *Eucryphia cordifolia* (Cunoniaceae), about 30 m tall, whose trunks and limbs are densely covered by epiphytes. Emergent trees were generally more than 350 years old (Gutiérrez et al. 2008).

The local climate is wet temperate, with a strong oceanic influence (di Castri and Hajek 1976). It has an annual rainfall of 2444 mm and a mean annual temperature of 10.7°C. The area of old-growth forest has remained untouched by significant human intervention for at least 450 years (Gutiérrez et al. 2008), and hence, the surrounding forest fragmentation is unlikely to have had a major effect on the epiphyte and invertebrate communities analyzed (e.g., Saunders et al. 1991, Barbosa and Marquet 2002). Moreover, because of their coastal location, these rain forests are largely free of atmospheric nutrients and pollutants of industrial origin,

and are likely to represent reference ecosystems for understanding biological processes in the absence of industrial impact (Hedin et al. 1995, Perakis and Hedin 2002). Gutiérrez et al. (2008) and Díaz et al. (2010) provide further details about the study area.

Experimental design

We selected four large, canopy emergent, *E. cordifolia* trees of similar physiognomy, which measured between 1.22 and 1.38 m in diameter at breast height (DBH) and reached 25 to 30 m in height; all four chosen trees supported healthy crown limbs and foliage. Focal trees were arranged in two pairs, each pair comprised of one control and one experimental tree. Trees in each pair were separated by less than 50 m, and both pairs were about 300 m apart. We randomly chose one tree in each pair to be subjected to manual removal of its entire epiphyte layer (Díaz et al. 2010). Selecting pairs in this way (stratified sampling) helped control unknown environmental variations (Green 1979). *Eucryphia cordifolia* represents an appropriate study model because it is one of the dominant trees in the Valdivian rain forest's canopy and results for this tree species may be representative of other species in this ecosystem type which are also densely covered by epiphytes. Tree density for *E. cordifolia* in the stand averages c. 70 individuals ha⁻¹, with a mean DBH of about 1 m (Gutiérrez et al. 2008, Díaz et al. 2010). Tree canopies were accessed using arborist techniques described in the Tree Climber Coalition's protocols (www.treeclimbingusa.com). First, using a slingshot we threw a small weight tied to a fishing line over a tall branch. Then, we replaced the fishing line with a stronger line and used it to pull the climbing rope over the limb. We climbed to this first limb using the single rope technique, and kept climbing on the upper limbs using the double rope technique, which allowed for safe movements across the entire crown. Once each tree was climbed for the first time and found to be appropriate for the study, we left a permanent line crossing over one branch near the treetop to provide access to the tree crown on later visits.

We removed the epiphytes from each experimental tree as completely as possible, using small axes, garden saws and knives. All the removed plant and soil material was placed in large 15 kg plastic bags, lowered to the ground, and

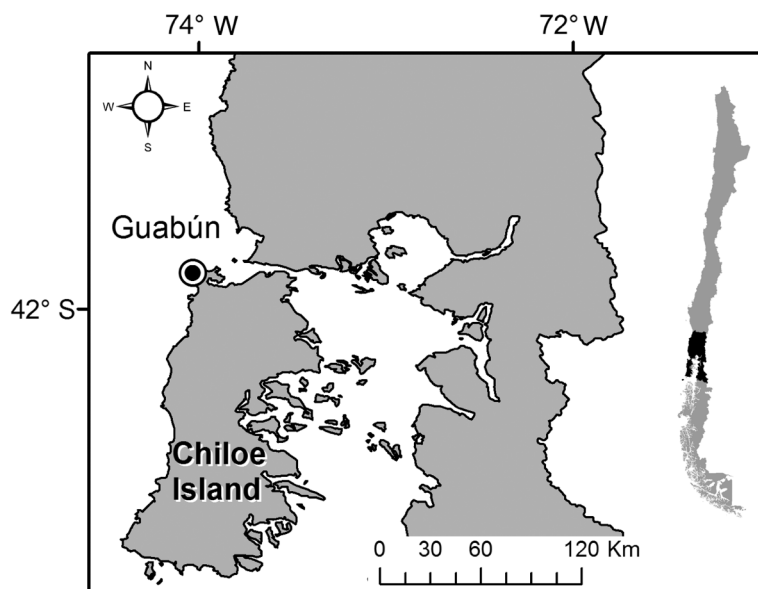


Fig. 1. Map of the study site showing the location of the coastal forest of Guabún, in Chiloé Island, southern Chile.

weighed. Díaz et al. (2010) provide further details on the removal techniques, sampling effort, and epiphyte communities. Finally, we characterized each tree's shape, estimated its bark surface area, foliage density, and epiphyte cover based on the protocols described by Van Pelt et al. (2004). In particular, we assessed the amount of foliage in the crown of each tree by defining a reference "foliar unit" representing a 2 m branch with all of its foliage (Van Pelt et al. 2004). This information was used to estimate invertebrate abundance in the crown (see below). The characterization of each tree sampled is presented in Appendix A.

Our experiment presented several limitations, such as the small sample size, the effect of the disturbance caused by the removal of epiphytes, and the effectiveness of traps to detect differences among treatments. Strong logistic restrictions limited our ability to increase the sample size of trees, because of the sampling effort involved and the remote location of pristine old-growth forests. However, previous studies have shown that morphospecies composition in trap catches was more strongly influenced by habitat type than by sampling technique (Missa et al. 2009). Accordingly, the results of our experiment, comparing large trees with intact epiphytes to large trees with epiphytes removed, should

effectively illustrate the differences attributable to the consequences of epiphyte removal. A detailed discussion of the validity of our experimental design and limitations of our sampling methods is given in Appendix B.

Invertebrate sampling on tree canopies

At the end of the austral summer (March) of 2006, four months after epiphytes were manually removed from the two experimental trees, we placed one flight interception trap (Basset et al. 1997) in the canopy of all four focal trees. Each trap was placed midway between the top of the crown and the lowest crown limbs and suspended from a branch with a pulley that allowed us to raise and lower the trap from/to the ground using a line. Traps were made of two intersecting mesh panels (1-m² each panel, 2 mm mesh size), with one funnel at the top and another at the bottom, so that flying insects were driven into collecting jars when moving up or down after entering the net. In addition, we placed two eclector traps per tree to catch walking or crawling invertebrates (Basset et al. 1997); one trap was placed on the main trunk about 13 m high and the second one on any accessible limb in the tree crown, about 16 m above the ground. Eclector traps consisted of a funnel made of a net

that was firmly tied on its side flush with the trunk surface, with a larger entrance (80 cm wide) at one end that led (for 80 cm) to a collecting jar at the narrower end of the funnel. Jars in all traps were filled with small pieces of paper to offer temporary refuge to invertebrates, and contained a plastic band impregnated with Diazinon, a poison that kills invertebrates as it slowly evaporates. Captured insects were collected every four days. All funnels were covered with plastic to prevent rain from filling the collecting jars. Sampling on both control and experimental trees was conducted on the same days; each sampling session lasted four consecutive days. Sampling sessions were spread out over the year, occurring every 4 to 5 weeks, resulting in a total of 12 surveys per tree between April, 2006 and May, 2007.

Invertebrate sampling on epiphytes

Additional sampling of canopy invertebrates was conducted on the two control trees with epiphytes intact. For this, we collected samples of about 300 cm³ of green tissues and arboreal soils of epiphytes from six locations in the crown of each tree. Samples were collected on one non-rainy day per season (winter, spring, summer and fall) from accessible limbs located between 8 and 17 m in the canopy, and stored in a closed plastic bag at a low temperature (<10°C), prior to transportation to the laboratory which occurred within 10 hours of the collection. Epiphyte samples were placed and maintained in Berlese funnels for four consecutive days until all invertebrates present were extracted (Basset et al. 1997).

Invertebrate abundance and biomass

We counted the number of collected individuals from each recognizable taxon (see below), and used estimates of invertebrate biomass as a measure of species abundance. Since one large individual accumulates more biomass than many smaller ones, we transformed individual numbers into biomass to standardize a common unit of abundance. To do this, we measured the body length of every individual invertebrate collected under a stereoscopic microscope with a graded ruler (0.2 mm precision). Measurements were taken from the top of the head to the end of the abdomen, avoiding antennas and/or reproduc-

tive structures. We then transformed this data into dry mass in grams, using allometric equations given by Sabo et al. (2002) and Collins (1992) for various orders of invertebrates. Snails were excluded from these analyses because of inconsistencies detected between the body length and the estimated biomass, based on the equations from Collins (1992), and because they represented a minor proportion of biomass samples (only 8 individual snails were collected, all with shells smaller than 2.5 mm).

Comparing invertebrates from tree crowns and epiphytes

An important challenge of this study was to differentiate between the invertebrate species found in the tree's crown and those directly associated with epiphytes. We estimated invertebrate biomass (1) for the entire tree crown and (2) for only the epiphyte loads in the following ways. (1) For the entire tree crown, we extrapolated flying insect numbers caught by the volume of one flight-interception trap in each survey to the volume of the entire tree crown. The panels of one flight interception trap had a volume of around 1 m³, which represented less than the volume occupied by two 'foliar units' of the tree crown (two branches plus their foliage; see above). Thus, we used two 'foliar units' as a conservative standard measure to estimate the tree crown contribution. We estimated the biomass of invertebrates per tree crown by multiplying the biomass of invertebrates captured by each interception trap times the number of 'foliar units' present within the crown, divided by two. Eclector traps were not used for these estimates because they included invertebrates that dwelled on epiphytes, and the number of captures was low. (2) To estimate the biomass of invertebrates associated with epiphytes, we first calculated the biomass of invertebrates present in each sample of epiphytic material collected. Later, we dried and weighed each sample of epiphytes, calculating the biomass of invertebrates per dry gram of epiphytic material. Finally, we multiplied this number by the total dry weight of the epiphytes per tree. This information also indicated how much invertebrate biomass was probably present in the epiphyte loads removed from experimental trees. Although this approach has some limitations,

such as the use of different methods for assessing invertebrate abundance in the tree crown and in epiphytes, estimates allow for a comparison of invertebrate biomass between control and experimental trees. We cannot use this data to obtain absolute estimates of invertebrate biomass for the forest stand.

Data analyses

Invertebrates collected by flight interception and eclector traps were classified using a microscope, first at a family level and then as morpho-species whenever possible. Specimens were stored for later determination by experts. Classification and storage followed the procedures described by CSIRO (1991). Invertebrates from epiphyte loads extracted with Berlese funnels, were classified at an order level because most of them differed from those in the canopy and the lack of taxonomic keys precluded more precise classification.

Species richness.—Invertebrate samples captured by each kind of trap were pooled for species richness analysis by tree. We computed sample-based rarefaction curves with 95% confidence intervals (Colwell et al. 2004) to compare differences in species richness (in our case morpho-species) between trees with and without epiphytes using EstimateS software version 7.5 (Colwell 2006). Rarefaction compares the total number of individuals counted with repeated random sampling to the total number of species found in those samplings (Colwell and Coddington 1994). Sample-based rarefaction permits comparison of species richness among taxonomic groups with different sample sizes using a Monte Carlo randomization procedure (Gotelli and Colwell 2001). Such procedures allowed for comparisons among samples with different total numbers of individuals. We also computed the total species richness estimator Chao2 using the software EstimateS. Chao2 is a robust sample-based species richness estimator that assesses the total number of species in the sample (in our case the tree crown), including those rare species not recorded, emphasizing the importance of species that occur only as singletons and doubletons in the species richness estimation (Magurran 2004). The Chao2 estimator provides the least biased estimate of species richness for small sample sizes (Colwell and Coddington 1994). Formulas

and descriptions of their performance can be found in Chao (1987) and Chazdon et al. (1998).

To assess the taxonomic similarity between the invertebrate faunas of trees with and without epiphytes, we analyzed the overlap in morpho-species composition using Sørensen's similarity index QS , defined as $QS = 2C/(A + B)$, where A and B are the total numbers of species in trees A and B , respectively, and C is the number of species shared by both trees. This index ranges from 0 (no species shared) to 1 (all species shared). We computed this index using the software EstimateS (Colwell 2006). We also assessed whether morpho-species present in trees that had their epiphytes removed were a subset of those in trees with epiphytes intact using the Nestedness Temperature Calculator program (<http://www.aics-research.com>). This program assesses the nestedness in species composition among samples based on presence/absence matrices using algorithms related to thermodynamic measures of order and disorder. Results were expressed as if it were a certain temperature T , where perfect nestedness is described as maximally cold ($T = 0^\circ$) and complete randomness is described as maximally hot ($T = 100^\circ$). A presence/absence matrix that contains both a certain degree of nestedness and randomness will show an intermediate temperature. Specific procedures for nestedness calculations are given by Atmar and Patterson (1993).

Abundance measurements.—We tested whether invertebrate abundances in the crown differed between trees with and without epiphytes over the year using Repeated Measures MANOVA, after testing for normality assumptions (Kolmogorov-Smirnov test, Mauchly's test for sphericity). When data did not meet assumptions, they were \log_{10} transformed (Zar 1996). This analysis was done only for invertebrates captured by flight interception and eclector traps, pooled by order and by tree. We pooled the results from both types of traps because most invertebrates (>75%) were captured by flight interception traps. Most invertebrates captured in eclector traps were a subset of those captured in flight interception traps. Finally, we analyzed whether invertebrate biomass from experimental trees covaried during the year using Pearson's correlation analysis (SPSS version 11.0, SPSS, Chicago, Illinois).

RESULTS

Epiphytes' contribution to invertebrate species richness in tree crowns

We collected a total of 4,520 individuals in the flight interception and elector traps. However, due to loss of body parts, we were able to classify only 3,837 individuals, for a total of 506 morpho-species. Rarefaction analysis showed that the number of morpho-species in large trees with epiphytes was nearly twice that found in similar trees after epiphyte removal (Fig. 2, Table 1). The number of morpho-species did not stabilize as the number of individuals trapped increased, indicating that we were able to record only a small subset of the total species richness present in the crown of each canopy emergent tree (Fig. 2). The estimator of total species-richness Chao2 also showed a higher species richness in trees

with epiphytes; these trees held 43 to 82% more morpho-species than experimental trees without epiphytes (Table 1). The similarity index of invertebrate species composition among sampled trees showed no clear pattern (Table 2). Trees with and without epiphytes shared between 40% ($n = 94$) and 48% ($n = 120$) of morpho-species (Table 2) and morpho-species found in experimental trees were not nested within control trees; no one tree or treatment contained a subset of the species from the other trees ($T > 60^\circ$).

Epiphytes' contribution to invertebrate biomass in tree crowns

The total biomass of invertebrates collected in flight interception and elector traps was significantly greater in trees with epiphytes than in those without epiphytes (repeated-measures MANOVA test $F_{1, 14} = 8.18$, $P = 0.013$; Table 3).

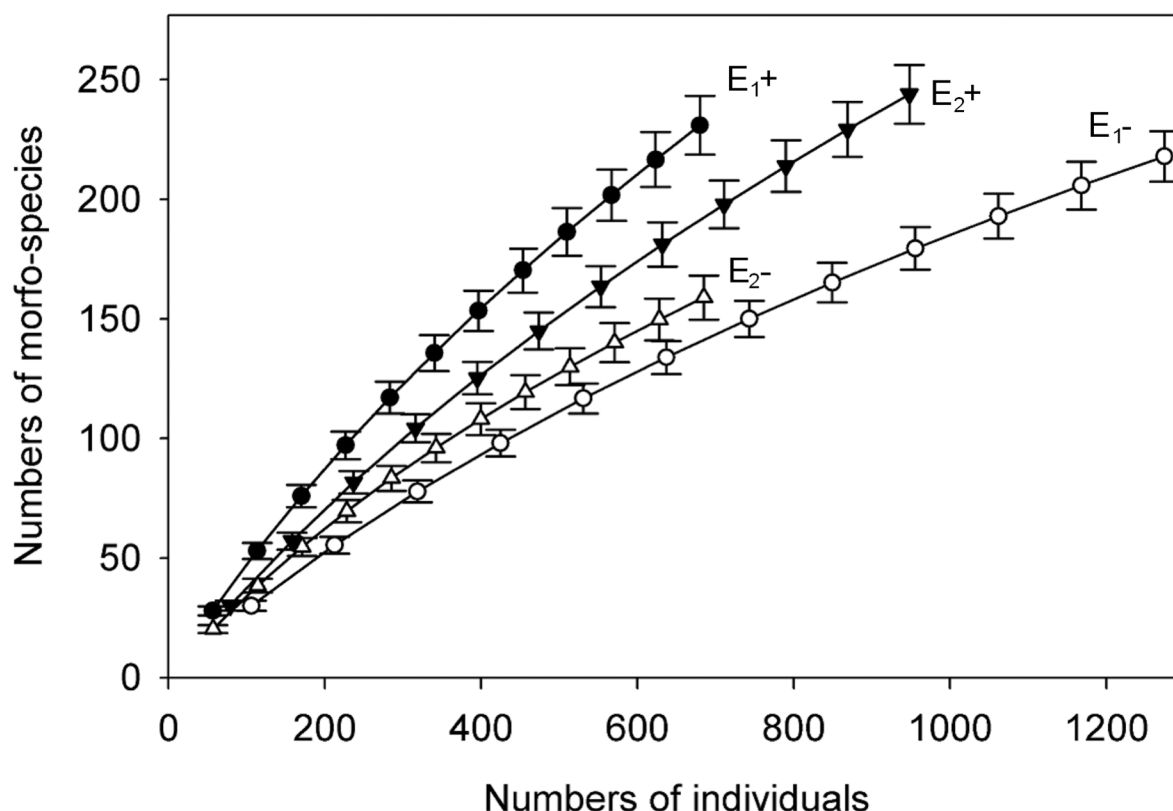


Fig. 2. Rarefaction analysis for the total number of invertebrate morpho-species as a function of the total number of individuals captured by the flight interception and elector traps placed in the canopy of four large canopy trees of *Eucryphia cordifolia*. E_{1+} and E_{2+} are trees with epiphytes intact, E_{1-} and E_{2-} are equivalent trees with their epiphytes removed. Subscripts refer to tree pairs one and two.

Table 1. Number of morpho-species recorded (± 1 SE, data from Rarefaction Analysis) and the total number of morpho-species predicted (based on the value of Chao 2) for invertebrates in the crown of equivalent trees with epiphytes (E_1+ and E_2+) and with epiphytes removed (E_1- and E_2-).

Metric	E_1+	E_2+	E_1-	E_2-
No. species recorded	231 \pm 12.19	244 \pm 12.26	218 \pm 10.55	159 \pm 9.21
No. species predicted	666	646	466	354
95% CI	507, 916	504, 865	376, 608	275, 488

Table 2. Sørensen's taxonomic similarity indices calculated for the number of individuals of each invertebrate morpho-species present in the crown of large *E. cordifolia* trees, for trees with epiphytes (E_1+ and E_2+) and trees from which epiphytes were experimentally removed (E_1- and E_2-). Values in parentheses are the numbers of shared morpho-species.

Treatment	E_2+	E_1-	E_2-
E_1+	0.476 (120)	0.466 (111)	0.404 (83)
E_2+		0.445 (108)	0.494 (103)
E_1-			0.480 (94)

Most invertebrates (92%) had small bodies, 8–16 mm long, and body masses of 0.02 to 0.10 g. Large differences in biomass occurred throughout the year, with higher invertebrate biomass in spring and summer (October–February) than in winter (June–July; repeated-measures MANOVA, within-subjects test $F_{1, 14} = 24$, $P < 0.001$; Fig. 3). Less seasonal variation was recorded for control trees with epiphytes intact. In trees which had their epiphytes removed, invertebrate biomass fluctuated markedly throughout the year,

but without a clear pattern (Fig. 3, Table 4). Pearson's correlation coefficients showed that monthly invertebrate biomass was similar between trees with epiphytes intact, but differed greatly between the trees without epiphytes, as well as between trees with and without epiphytes (Fig. 3, Table 4).

Invertebrate biomass varied strongly along the survey, depending on the taxonomic order (Table 3). A Tukey's post-hoc comparison identified two main groups. The first group, composed of Hemiptera and Coleoptera, was the most abundant overall; these taxa exhibited pronounced increases (outbreaks) in abundance during spring and summer (Appendix C). The second group included Aracnida, Diptera, Lepidoptera and Hymenoptera which had lower abundances overall, and exhibited less pronounced increases during late summer (Tukey's HSD mean difference < -0.798 , $P < 0.02$). Post-hoc tests did not identify any invertebrate order of crown invertebrates that was markedly affected by the removal of epiphytes (Appendix C).

Invertebrate biomass in epiphyte loads

The biomass of invertebrates collected in the Berlese funnels varied among surveys from 0.02 g to 2.42 g of invertebrates per kg of epiphytic dry mass, averaging 1.0 ± 0.5 g of invertebrates/kg of epiphytic dry mass. Thus, as trees sampled supported 97 and 209 kg of epiphytes, then they may hold between 97 ± 48 g and 209 ± 105 g of invertebrate biomass. Most of the invertebrates collected weighed between 0.04 and 0.08 g and animals in this mass range accounted for 92% of the total invertebrates dwelling in epiphyte loads.

The dominant groups of invertebrates in epiphytes were Miriapoda (mainly Chilopoda or centipedes), Oligochaeta (earthworms) and

Table 3. Repeated-measures MANOVA on biomass (g) of different invertebrate orders recorded in *E. cordifolia* trees by treatment: with epiphytes intact and with epiphytes removed.

Source	Type III SS	df	Mean square	F	P
Intercept	0.620	1	0.09141	128.6	<0.001
Treatment	0.00581	1	0.00581	8.186	0.013
Orders	0.05833	6	0.00972	13.67	<0.001
Treatment \times Orders	0.021	6	0.00351	4.94	0.007
Error	0.00952	14	0.00071		

Note: SS is sum of squares.

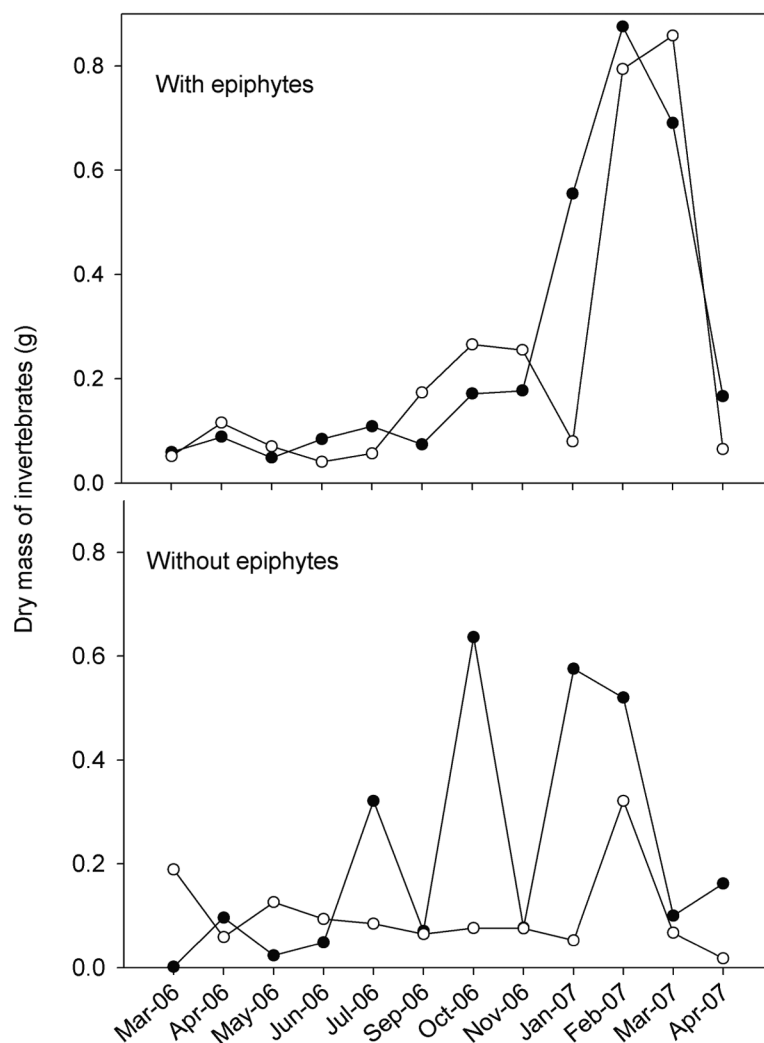


Fig. 3. Estimated biomass (g of dry mass per survey) of invertebrates captured in the crown of large *Eucryphia cordifolia* trees with epiphytes intact and with epiphytes removed in the old-growth forest of Guabún, Chiloe Island, Chile. Data are for the period between the austral fall of 2006 to the austral fall of 2007. Solid circles are control and manipulated trees in pair one, and open circles are control and manipulated trees in pair two.

Isopoda (pillbugs; Appendix D). Spiders and pseudoscorpions accounted for 2% of the invertebrate biomass (Appendix D). Ants were very rare (only 17 individuals captured), and close to 6% of the invertebrate biomass belonged to unidentified larvae. Many tiny mites (<0.5 mm body length) were found, but they represented a small biomass, and hence were excluded from the analyses.

Contrasts between invertebrate biomass and richness in tree crown vs. epiphytes

Invertebrate biomass associated with epiphytes was up to two orders of magnitude greater than estimates obtained for the crown foliage (Table 5). Epiphytes held as many or more species of spiders (predators) than the tree crown, and included a high biomass of centipedes (predators) and Oligochaeta (detritivore worms), both absent in the tree crown foliage. In contrast to the tree crown, epiphytes were inhabited by fewer herbivores (e.g., moth larvae,

Table 4. Pearson's correlation coefficients for the biomass of canopy invertebrates present in *E. cordifolia* trees with epiphytes (E_1+ and E_2+) and with epiphytes experimentally removed (E_1- and E_2-) for one year of sampling. ** $P < 0.005$.

Treatment	E_1-	E_2+	E_2-
E_1+	0.509	0.834**	0.451
E_1-		0.231	0.180
E_2+			0.445

and others), accounting for less than 1% of the entire epiphyte invertebrate fauna. Epiphyte loads contained the majority of the biomass of functional groups not otherwise present in the forest canopy.

DISCUSSION

Links between epiphytes and canopy invertebrates

Epiphytes represent a conspicuous structural component of the canopy of rain forests, increasingly abundant in larger and older trees (Díaz et al. 2010). Our field experiment shows clear links among structural elements (the epiphytes) and species richness and composition (the diversity of invertebrates) in the forest canopy. Epiphytes elevated the overall species count in the whole tree crown, by adding entire assemblages of

morpho-species restricted to epiphyte loads. Epiphytic invertebrates markedly increased the total biomass of invertebrates in control trees, and, at the same time, the presence of epiphytes buffered the fluctuations of invertebrate abundance in the tree crown. Our study links the structure and composition of biodiversity with ecological functions in old-growth forests, since epiphytes added new functional groups of invertebrates to the trophic web in the forest canopy. Added functional groups are detritivores and predators. These links may become increasingly strong as host trees (which are another structural component) become older.

Epiphytes and invertebrate species richness and abundance

Observational and comparative studies conducted in tropical forests of Panama, Costa Rica and Borneo showed that epiphytes support a high species richness and abundance of unique invertebrate groups, such as Miriapoda, Isopoda and Oligochaeta (Stuntz et al. 2002, Yanoviak et al. 2003, Elwood and Foster 2004). The remarkable abundance of invertebrates associated with the epiphyte layer seems to be a common pattern in many forest ecosystems. For instance, in shaded coffee plantations in Mexico, trees with epiphytes had 90% greater densities of invertebrates than trees without epiphytes (Cruz-Angón

Table 5. Invertebrate biomass (g dry mass) estimated for the whole tree crown of *E. cordifolia*, and for the epiphytic loads. Data are shown for trees with epiphytes intact (E_1+ and E_2+) and for trees from which epiphytes were experimentally removed (E_1- and E_2-).

Taxa	Food habit	E_1+	E_2+	E_1-	E_2-
Tree crown					
Aranae	Predator	3.7	1.0	1.9	0.5
Coleoptera	Generalists	9.0	2.4	4.5	1.1
Hemiptera	Herbivore	5.9	1.6	3.0	0.7
Hymenoptera	Generalists	1.3	0.3	0.6	0.2
Diptera	Generalists	3.9	1.0	2.0	0.5
Lepidoptera	Pollinator	2.8	0.8	1.4	0.4
Other	Generalists	2.2	0.6	1.1	0.3
Total		28.8	7.7	14.5	3.6
Epiphytes					
Aranae	Predators	6.5	0.8	0	0
Coleoptera	Generalists	3.0	0.8	0	0
Miriapoda	Predators	64.4	32.9	0	0
Oligochaeta	Detritivores	97.9	37.5	0	0
Isopoda	Detritivores	15.7	32.1	0	0
Larvae	Generalists	10.9	5.6	0	0
Other		15.7	8.4	0	0
Total		214.0	118.1	0	0

et al. 2009). In the tropical Dipterocarp forest of Borneo, a single large epiphytic fern supported 88 ± 14 g of invertebrate biomass, compared to the 86 ± 18 g held by the entire tree crown (Ellwood and Foster 2004). In the forest of Chiloé, invertebrate biomass in the crown of the trees without epiphytes ranged between 3.6 to 28 g per tree, much lower than the biomass of invertebrates dwelling in the epiphyte layer, which ranged from 97 to 209 g per tree. Accordingly, the vast majority of invertebrates living in a tree crown are epiphyte dwellers, and this pattern seems to occur elsewhere.

Possible functional role of invertebrates supported by epiphytes

A central finding of our study was that the removal of epiphytes signified the loss of major functional groups of invertebrates associated exclusively with epiphytes. Clearly, epiphytes add resources (organic detritus, green tissues) and modulate the local microclimate, particularly moisture, thus providing suitable habitat for a wide variety of invertebrates across a range of functional groups, from herbivores and predators to detritivores (e.g., Stork et al. 1997, Amédégnato 2003). However, it appears that unlike the canopy foliage, epiphytes favor predators and detritivores over herbivores. In our study, epiphyte loads supported a significantly higher biomass of detritivores and predators, but a lower biomass of herbivores. Stuntz et al. (2002) and Yanoviak et al. (2004) derived similar conclusions regarding invertebrate assemblages associated with epiphytes in tropical forests.

Consequently, we hypothesize that the presence of epiphytic detritivores provides an abundant and stable food source for predators and, in turn, epiphyte-based predators likely exert an important control of foliage herbivores. Increasing numbers of predators in forest ecosystems commonly depress foliage herbivores (Van Bael et al. 2008, Ruiz et al. 2009, Mooney et al. 2010). Moreover, predators of herbivores can be enhanced by detritus-based prey subsidies. For example, in stream ecosystems, emergence of aquatic invertebrate prey subsidizes terrestrial predators that then feed more heavily on herbivorous larvae on trees, such as insectivorous birds in Japan (Murakami and Nakano 2002) or

spiders in Germany (Henschel 2004). Such detritus-based strengthening of predation may come from outside the system (e.g., Polis and Hurd 1996). However, because epiphytes trap and build their own detritus and soil (Enloe et al. 2006, Díaz et al. 2010), prey subsidies to predators in this case, possibly in the form of worms and other detritus-feeding insects in epiphytic soil, can originate from within the system.

Control trees with epiphytes intact showed lower monthly variability of invertebrate biomass than trees which had their epiphytes removed, suggesting that epiphyte cover not only enhances invertebrate abundance in the canopy, but also downsizes fluctuations of invertebrate biomass over the annual cycle (see Table 4, Fig. 3). This moderating influence of epiphytes could be extremely important from a functional perspective, since epiphytes could thus provide a fairly stable food supply to predators of foliage herbivores all year round. In this way, predators may control seasonal outbreaks of herbivores, impeding or diminishing their undesirable effects on tree foliage. Evidence supporting this hypothesis from our data is weak, since the abundance of insect herbivores was highly variable. However, Díaz (2009) recorded a greater number of visits by insectivorous birds to the trees with epiphytes. Birds can limit herbivory directly by feeding on invertebrates on the tree (Mazia et al. 2004), while epiphytes may indirectly control herbivory by subsidizing insect-feeding birds. We are optimistic about testing this hypothesis given the tractability of the epiphytic subsystem in the forest canopy. As shown here, epiphytes allow for experimental manipulations that could lead to deeper understanding of complex food webs and functional dynamics.

Large old trees, epiphytes and invertebrates: structurally relevant species for biodiversity conservation

The oldest and largest trees in the canopy are important structural components of forest ecosystems (Franklin et al. 1981, Berg et al. 1994). The structure created by trees becomes increasingly more relevant with age, supporting additional structures created by associated organisms, such as epiphytes, thus modifying

habitats to support greater species richness, abundance and functional species diversity, such as a large diversity of invertebrates and the inclusion of predators and detritivores in the forest canopy. In the old-growth forest of Chiloé, 350 year old canopy trees of *E. cordifolia* supported an outstanding biomass and diversity of epiphytes, which were absent from younger trees (Díaz et al. 2010). Consequently, species diversity and ecological functions supported by the epiphytes in older trees should be much broader than in the case of younger trees that lack these epiphytes.

The few tree species that can become old enough to support species diversity can be considered “structurally relevant species”, because their presence strongly influences species composition and ecological functions of many rain forests ecosystems. For instance, the nutrient cycling and the control of herbivory in the canopy may be largely under control by functional groups associated with epiphytes, such as lichens (Benner et al. 2007), mosses (Lindo and Whiteley 2011), detritivorous and predatory invertebrates (e.g., Losey and Vaughan 2006, this study), or vertebrates (Cruz-Angón and Greenberg 2005). Old trees provide key habitat for cavity nesting vertebrates, otherwise absent or scarce in the forest (Cockle et al. 2011). In conclusion, large trees with epiphytes seem to be fundamental in defining forest biodiversity and ecosystem processes, and because of the life span of trees, their importance can extend for hundreds or even thousands of years. These trees, with their epiphytes intact, are evidently non-replaceable, but they are often neglected in many areas of forest research and conservation (Hazell and Gustafsson 1999, Löhmus and Löhmus 2010, Ulyshen 2011). Because of their uniqueness, these structurally relevant trees should be of central priority for biodiversity conservation, particularly in managed forest ecosystems and in human dominated landscapes (Franklin et al. 1981, Díaz et al. 2010, Lindo and Whiteley 2011, Ulyshen 2011).

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SUPPLEMENTAL MATERIAL

APPENDIX A

Characterization of the studied trees

The four large *E. cordifolia* trees selected for the study of epiphytes and associated invertebrate assemblages measured between 1.22 and 1.38 m in diameter at breast height (DBH), with estimated heights between 25 and 30 m. Selected trees had healthy foliage and crown limbs, and were similarly covered by a dense layer of epiphytes, dominated by bromeliads and ferns, but including individuals of the hemi-epiphytic tree *Raukaua laetevirens* (Araliaceae). This hemi-epiphyte is typically restricted to the crown of large canopy emergent trees. The exposed bark

surface of each tree was estimated by measuring the length, diameter, cardinal direction (North, South, East, West) and height along the vertical profile of all branches, from the base of the trunk to the treetop (after Van Pelt et al. 2004). With the stem diameter and length data, we estimated the exposed surface and volume of each canopy branch and, in turn, the area and volume of the whole tree crown, assuming each limb measured had a cylindrical shape. Estimation of epiphyte biomass of each control tree was based on the data of epiphyte biomass removed from each experimental tree. First, we divided the total epiphyte biomass removed by the exposed bark surface of each respective manipulated tree (in m²). This provided an estimate of epiphyte biomass per m² of bark surface, one value per tree for the two experimental trees. We averaged the value of the two trees, and multiplied it by the total bark surface of each control tree, obtaining an estimate of its total epiphyte biomass. Finally, we assessed the amount of each tree's crown foliage by defining a reference “foliar unit”, equivalent to a 2 m long branch with all of its foliage intact (Van Pelt et al. 2004).

Total epiphyte biomass of trees from which epiphytes were removed (excluding one hemi-epiphyte not removed) varied between 134 and 144 kg of dry mass, with 70% contributed by epiphytic soil and roots, and the remaining 30%

Table A1. Main features of the *E. cordifolia* trees selected for this study in the coastal forest of Guabún, Chiloé Island, Chile. Biomass estimates are expressed as dry mass. DBH = diameter at the breast height. Control trees with epiphytes intact are noted as E₁+ and E₂+; trees with epiphytes removed are noted as E₁– and E₂–.

Feature	E ₁ +	E ₂ +	E ₁ –	E ₂ –
Tree DBH (m)	1.38	1.23	1.33	1.23
Tree height (m)	30	25	30	25
Bark surface (m ²)	213	98	145	106
Number of foliage units	351	94	144	107
Epiphytic biomass removed (kg)	None	None	144	133
Epiphytic biomass remaining (kg)	209	97	<20	<20

by the green leaves and stems of vascular epiphytes (Díaz et al. 2010). Based on these numbers, the total epiphyte biomass (dry weight) per unit of bark surface was $0.98 \pm 0.27 \text{ kg/m}^2$. Based on this value, we estimated that control trees held between 97 and 209 kg of epiphytic biomass each.

APPENDIX B

Methodological limitations

Our study presented several methodological limitations, which are unlikely to invalidate our main conclusions. However, they are important for understanding how general our results can be. The first limitation is the low sample size, with only two replicates (whole trees) per treatment, due to the logistic difficulty of removing all epiphytes from large, emergent canopy trees in remote old-growth forests (cf. Díaz et al. 2010). The low sample size limited the opportunities to generalize, but our results showed very clear patterns and are in agreement with other studies of epiphytes, suggesting that despite the small sample size the effects of epiphytes on invertebrate diversity and biomass is strong in both tropical and temperate rainforests.

A second limitation is due to the disturbance caused to experimental trees during the removal of epiphytes. The lower richness of invertebrate morpho-species recorded in trees which had their epiphytes removed could possibly be related to the disturbance caused by the manual removal, rather than the lack of epiphytes alone. To attenuate the effect of the removal, we started surveying invertebrates four months after the manipulation of epiphytes to allow for the recovery of the invertebrate assemblages. However, we had no prior information to assure that four months was enough time for this recovery. In our surveys, trees with epiphytes often had higher species richness than trees without epiphytes, even one year after manual extirpation,

proving that the lack of epiphytes (and not the disturbance due to removal) explained the lower insect species richness. Finally, a third limitation is related to the types of traps used to collect both flying and walking invertebrates. Interception traps had not been previously used for a comprehensive estimate of invertebrates inhabiting the tree crown. The methodology used to estimate the whole invertebrate community in tree crowns (extrapolation of interception trap volume to the entire crown volume) might underestimate invertebrate richness and abundance by some orders of magnitude in light of other studies (e.g., Battistola et al. 2007). This method may also overestimate insect abundance since it captures a small volume of invertebrates moving in the tree crown. However, the study of Missa et al. (2009) showed that morpho-species composition in trap catches was far more influenced by habitat than by the sampling method. That is, differences in trap efficiency between habitats did not lessen the ability of the method to detect real habitat-based contrasts. Here we experimentally created two contrasting habitats, tree crowns with over 500 kg (fresh weight) of epiphytes versus tree crowns which had their epiphytes manually removed. Moreover, our findings are consistent with previously reported effects of epiphytes on invertebrate assemblage structure (e.g., Ellwood and Foster 2004).

Despite such limitations, we present strong evidence that epiphytes hold a conspicuous diversity and biomass of invertebrates, which greatly influence the overall abundance and composition of invertebrates in the forest canopy. Consequently, it is likely that improvements in methods will not change our general conclusion, showing that epiphyte loads are a structural component of forest canopies which determine the composition of invertebrates and their ecological functions.

APPENDIX C

Table C1. Total biomass (g) and number of individuals (in parentheses) of the main invertebrate orders collected by flight interception and eclector traps placed in the crown of large *E. cordifolia* trees with epiphytes intact (E_1+ and E_2+) and with epiphytes manually removed (E_1- and E_2-).

Taxa	E_1+	E_2+	E_1-	E_2-	Total
Aranae	0.26 (111)	0.52 (170)	0.61 (115)	0.23 (66)	1.64 (462)
Coleoptera	1.06 (250)	1.15 (251)	1.20 (218)	0.64 (185)	4.06 (904)
Diptera	0.39 (386)	0.35 (649)	0.72 (920)	0.29 (452)	1.76 (2407)
Hemiptera	0.88 (16)	1.20 (24)	0.32 (11)	0.28 (4)	2.69 (55)
Hymenoptera	0.29 (51)	0.13 (45)	0.13 (43)	0.03 (13)	0.58 (152)
Lepidoptera	0.27 (57)	0.26 (72)	0.36 (83)	0.37 (84)	1.27 (296)
Oligochaeta	...	0.07 (1)	0.22 (1)	...	0.29 (4)
Other	0.25 (57)	0.14 (77)	0.13 (48)	0.12 (41)	0.653 (223)
Total	3.41 (928)	3.84 (1291)	3.71 (1448)	1.99 (845)	12.92 (4512)

APPENDIX D

Table D1. Total biomass (g dry weight) and number of individuals (in parentheses) of the main invertebrate orders collected from epiphyte loads in the canopy of two undisturbed, large *E. cordifolia* trees.

Taxa	<i>E. cordifolia</i> 1	<i>E. cordifolia</i> 2	Total	%
Aranae	0.015 (24)	0.003 (18)	0.022(42)	1.6
Pseudoscorpionidae	0.002 (23)	0.002 (31)	0.006 (54)	0.4
Isopoda	0.036 (26)	0.126 (103)	0.169 (129)	12.0
Miriapoda	0.148 (10)	0.129 (60)	0.602 (70)	42.9
Coleoptera	0.007 (20)	0.003(7)	0.019 (27)	1.4
Hemiptera	0.002 (1)	0.000 (0)	0.003 (1)	0.2
Homoptera	0.002 (7)	0.001 (13)	0.004 (20)	0.3
Hymenoptera	0.020(7)	0.013 (10)	0.049 (17)	3.5
Larvae	0.025(39)	0.022 (21)	0.128(60)	9.1
Oligochaeta	0.225(7)	0.147 (5)	0.372 (12)	26.5
Opilionida	0.000(1)	0.001 (7)	0.001 (8)	0.07
Other	0.010(17)	0.016 (32)	0.028 (49)	1.9
Total	0.491(182)	0.462 (307)	1.404 (489)	100