Complexity of leaf miner–parasitoid food webs declines with canopy height in Patagonian beech forests

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Abstract. 1. Consumer–resource species interactions form complex, dynamic networks, which may exhibit structural heterogeneity at various scales. This study set out to address whether host–parasitoid food web size and topology vary across forest canopy strata, and to what extent foliar resources and species abundances account for vertical patterns in network structure.

2. The vertical stratification of leaf miner–parasitoid food webs was examined in two monotypic beech (*Nothofagus pumilio*) forests in northern Patagonia, Argentina. Quantitative food webs were constructed for separate canopy layers by sampling foliage from three tree-height classes at 0.5–1, 2–3 and 5–6 m above ground.

3. Leaf miner abundance per unit leaf mass and foliar damage (%) did not differ across strata, although foliage quality and quantity increased from the understorey to the upper canopy. Parasitism rates and food web complexity decreased with canopy height, as reflected by reduced linkage richness, linkage density, mean interaction strength, and host vulnerability.

4. Null model analyses revealed that food web metrics, especially in the upper canopy, were often lower than expected when compared with randomly structured networks. Overall, these patterns held for two forests differing in vertical structure and in dominant miner morphotype and parasitoid species.

5. These results suggest that vertical declines in network complexity may be driven by the parasitoids' limited functional response to host abundance and dispersal from pupation sites in the forest floor. A broader constraint on food web structure seemed to be imposed by host–parasitoid trait matching, a reflection of large-scale assembly processes.

Key words. Herbivory, network structure, null model, parasitism, stratification.

Introduction

All species are embedded in complex, dynamic interaction networks (Bascompte, 2009), which may exhibit variation in size and topology at various scales (Aizen *et al.*, 2012; Morris *et al.*, 2014; Valiente-Banuet *et al.*, 2015). Recent work has focused on the spatial heterogeneity of food web structure along human-modified (Memmott *et al.*, 2006; Tylianakis *et al.*, 2007) or natural (Morris *et al.*, 2015) habitat gradients. In contrast, the structural heterogeneity of antagonistic or mutualistic networks

within particular habitats has received less attention (Paniagua *et al.*, 2009; Donatti *et al.*, 2011; Schleuning *et al.*, 2011; Morris *et al.*, 2015). A better understanding of how food web attributes vary both within and among sites may help to anticipate effects from anthropogenic disturbances on biodiversity and ecosystem functioning (Tylianakis *et al.*, 2007; Thompson *et al.*, 2012).

Insect herbivores and their parasitoids account for a major fraction of the Earth's biodiversity (Price, 2002; Lewinsohn & Roslin, 2008), and represent the main consumers in most forest canopies (Coley & Barone, 1996). Vertical heterogeneity in forest structure has long been thought to maintain species diversity by providing a template for niche partitioning (MacArthur, 1972; Lewinsohn & Roslin, 2008). Despite substantial evidence

that insect abundance and diversity often vary with canopy height (e.g. Basset *et al.*, 1992, 2003; Brown *et al.*, 1997; Ribeiro & Basset, 2007; Sobek *et al.*, 2009; Ulyshen, 2011), little is known about the vertical stratification of arthropod food webs (Morris *et al.*, 2015). Only two studies in tropical forests have examined the structure of insect food webs across canopy strata. In a dry forest in Panamá, Paniagua *et al.* (2009) found that host specificity for gall maker–parasitoid webs was greater in the canopy, whereas understorey plants supported a larger, more connected food web. However, for cavity-nesting Hymenoptera and their parasitoids in Australian rainforests, Morris *et al.* (2015) observed no significant change in species diversity, parasitism rate or food web properties between the canopy and the understorey. Thus, whether and how food web structure should be expected to change within forest canopies remain unclear.

The structure of interaction networks results from multiple processes affecting species richness, composition, and relative abundance in local communities (Vázquez *et al.*, 2009a; Pillai *et al.*, 2011). Canopy structure may influence insect food web assembly through vertical changes in biotic or abiotic factors that differentially affect species' performance and trophic interactions (Price, 2002; Basset *et al.*, 2003; Ulyshen, 2011). In mature forest stands, foliage cover increases from lower to upper strata, determining the amount of resources and habitat available to folivorous insects and their natural enemies (Van Bael *et al.*, 2003). Foliar traits including specific leaf area, toughness, and defensive chemicals have been found to differ between the understorey and the canopy, and to correlate with vertical patterns of insect abundance and herbivory (Coley & Barone, 1996; Ribeiro & Basset, 2007). Top-down effects from predation or parasitism may also vary with canopy height (Van Bael *et al.*, 2003; Ribeiro & Basset, 2007; Sobek *et al.*, 2009), potentially affecting herbivore assemblages and whole food web structure (Paniagua *et al.*, 2009).

In addition, forest vertical structure creates microclimatic gradients that result from increased solar radiation, temperature, and wind speed towards the upper canopy (Parker, 1995; Ozanne *et al.*, 2003). Such abiotic heterogeneity can strongly affect the distribution of insect herbivores and carnivores (Coley & Barone, 1996; Ribeiro & Basset, 2007; Ulyshen, 2011), and may thus impinge on food web dynamics. Furthermore, community patterns can be generated by dispersal dynamics (Levine, 2003). High colonisation rates enhance the potential for interspecific interactions during food web assembly (Fahimipour & Hein, 2014), and may affect not only species diversity, but also network attributes such as generality and richness of feeding links (Murakami *et al.*, 2008; Pillai *et al.*, 2011). Hence, within-habitat food web structure could be related more to distance from oviposition or pupation sites than to spatial variation in abiotic factors, food availability, or predation (Brown *et al.*, 1997).

In general, the realised structure of bipartite networks (e.g. host–parasitoid webs) is affected by two sets of processes that reflect the roles of species abundance and species identity during community assembly (Vázquez *et al.*, 2005, 2009a). First, trophic interactions may arise from random individual encounters, so that interaction probabilities will depend solely on species' relative abundances, a process termed 'interaction

neutrality' (Vázquez *et al.*, 2009a). Second, whether a pair of consumer and prey species engage in a trophic interaction depends on their particular phenotypes or specific identities, a process referred to as 'trait matching' (Jordano *et al.*, 2003; Vázquez *et al.*, 2009a). There can be morphological, behavioural or phenological constraints for certain species to interact with each other, which will affect the observed network complexity (Vázquez, 2005; Petchey *et al.*, 2008; Olesen *et al.*, 2010). Such trait-based limitations to food web assembly may reflect the influence of ecological determinants of local species composition (Pillai *et al.*, 2011) or, ultimately, evolutionary constraints associated with the phylogenetic history of the community (Lepänen *et al.*, 2013). Thus, both the abundance and identity of insect species reaching different forest strata may be responsible for vertical patterns in network complexity.

In this study, we examined the vertical stratification of leaf miner–parasitoid food webs in cool-temperate forests of southern South America. Host–parasitoid systems are ideal for assessing heterogeneity in food web structure, because trophic links can be readily quantified (e.g. Lewis *et al.*, 2002; Valladares *et al.*, 2012). The small size and low mobility of the focal insects imply that species' abundances may be strongly affected by dispersal dynamics (Murakami *et al.*, 2008), as well as by subtle changes in biotic and abiotic factors (Price, 2002). Specifically, we aimed to determine: (i) whether host–parasitoid food web size and topology differed across vertical forest strata; (ii) how vertical shifts in host abundance and parasitism covaried with tree foliage cover and leaf traits; and (iii) the extent to which species abundance versus identity accounted for the observed network structure in separate forest strata. We hypothesised that vertical changes in foliage cover drive insect abundance and food web structure by determining the amount of habitat and food resources available to leaf miners and their parasitoids. We thus expected food web complexity to increase with foliage cover from the lower to the upper canopy strata. In addition, as shown for other bipartite networks (Vázquez *et al.*, 2005, 2009a), we expected species abundance (or interaction neutrality) to influence web attributes in different canopy strata.

We studied interactions between parasitic wasps and leaf-mining insects on *Nothofagus pumilio* (Poepp. & Endl.) Krasser, the dominant tree in subalpine forests of northern Patagonia, Argentina. The monotypic nature of these forests allowed us to focus on vertical food web patterns without the confounding effect of plant composition (cf. Paniagua *et al.*, 2009). We measured leaf miner abundance, leaf miner parasitism, foliar cover, and leaf traits for *N. pumilio* trees in three separate canopy strata. We then generated quantitative food webs and weighted network metrics (Bersier *et al.*, 2002; Dormann *et al.*, 2009) for each canopy layer, and used a null model approach to test for the role of species abundance in determining food web attributes (Vázquez *et al.*, 2005).

Methods

Study sites

The study was performed in two monotypic forests located 71 km apart in the northern Patagonian Andes, Argentina. The

main canopy species at both sites was the tall deciduous *N. pumilio*, which in this region dominates the subalpine zone between 1000 and 1600 m above sea level (a.s.l.) (Veblen *et al.*, 1996). Study sites were located at 1270 m a.s.l. in Paso Córdoba (40°35′42″S, 71°08′44″W; hereafter 'PC'), and at 1230 m a.s.l. in Valle Challhuaco (41°14′58″S, 71°16′59″W; hereafter 'CH'). The soils are shallow Andisols developed on recent volcanic ash deposits. The climate is cold-temperate, with wet winters and dry summers; most precipitation falls during autumn and winter (April–September). The region encompasses a steep west–east moisture gradient created by the rainshadow of the Andes (Veblen *et al.*, 1996). Study sites were representative of *N. pumilio* 'dry' forests with 800 (PC) and 1200 mm (CH) of annual precipitation (Mazía *et al.*, 2012). Both sites were free of large-scale disturbances (e.g. wildfires) for more than 100 years.

The study forests harbour various folivorous guilds, including leaf miners, leaf tiers, gall makers, and exophytic chewers and sap suckers (McQuillan, 1993; Mazía *et al.*, 2004, 2012). Leaf miners constitute the dominant guild, producing 50–80% of the leaf area damage in dry forests (Mazía *et al.*, 2004, 2012). Mining insects comprise species in the Hymenoptera and Lepidoptera, forming distinctive mine shapes. Leaf miners overwinter as pupae in the soil and emerge in October–December (spring) during the early stages of the growing season (October–April). Most leaf miner feeding occurs during December–January (summer), until the larvae vacate the leaves and drop to the ground in search of pupation sites. All leaf miners undergo one generation per growing season. Parasitoids emerge in spring from the leaf-litter layer and attack *N. pumilio* leaf miners from late spring to mid summer. The successful parasitoid pupates inside the mine and falls to the ground with the litter shed at the end of the season (April–May).

Vertical canopy structure and tree foliage quality

N. pumilio forests present a discontinuous vertical structure with three well-defined canopy layers (Paritsis *et al.*, 2015). Vertical strata correspond to different tree-height classes: the understorey of tree seedlings (0.5–2 m height, 1–3 cm basal diameter), a midstorey of saplings [∼3–5 m height, 3–10 cm diameter at breast height (dbh)] and the overstorey of adult trees (8–20 m height, 10–50 cm dbh). To describe vertical changes in the amount of foliage available to canopy insects, we surveyed density, basal area, and foliar cover of *N. pumilio* trees in each height class. Stand structure was measured along 50 m long × 4 m wide parallel transects placed at ∼20 m intervals [*n* = 6 (CH) and 4 (PC) transects]. Sampling effort was greater in CH to account for the scarcer numbers of tree seedlings and saplings. We counted all *N. pumilio* seedlings, saplings, and adult trees present within the 200 m^2 plot delimited by each belt transect, and measured basal diameter for seedlings and stem dbh for saplings and adults to calculate basal area (m^2 ha^{-1}) for each height class. We placed a 4 m^2 plot at 10 regularly spaced points along each transect to estimate the projected ground cover (%) of seedlings and saplings. Foliage cover of adult trees was measured at each sampling point using a hand-held densitometer. Differences across canopy layers (three levels)

and sites (two levels) were tested using type III ANOVAS. Cover was arcsine-square-root-transformed, and basal area and density were log-transformed; models met the assumptions of normality (cumulative residual plots) and variance homogeneity (Levene's test, $P = 0.05$).

To assess vertical changes in foliage quality, we measured physical and chemical leaf traits (Mazía *et al.*, 2012) for samples collected from each canopy layer during January 2011. In each forest, 10 *N. pumilio* trees were selected from each tree-height class. Due to limited accessibility, samples for adult trees (max. height = 8–20 m) were taken from the lower to the middle section of the crown (5–6 m). We harvested one branch from adult trees and saplings (2–3 m) using a pole pruner; seedling samples (∼1 m) were clipped by hand. Leaf size (cm^2), toughness (punch resistance, g mm^{-2}), specific leaf area (mm^2 mg^{-1}), water content (% fresh mass), N content (%), and total phenols (mg gallic acid g^{-1} dry mass) were measured for subsamples of 30 nonsenescing, undamaged leaves using protocols described in Mazía *et al.* (2004, 2012). Differences in foliar traits across strata (three levels) and sites (two levels) were tested with type III, two-way ANOVAS on untransformed data.

Vertical patterns of herbivory and parasitism

Insect sampling was performed in early January 2011 during the peak season of leaf miner activity (Mazía *et al.*, 2012). In each forest, we randomly selected 10 trees from each height class (=canopy layer) within a 2 ha stand. We ensured that trees selected from different height classes were spatially interspersed and separated by at least 10 m. Seedling leaves were collected by clipping two branches at ∼0.5–1 m height. For each sapling and adult tree, we harvested the distal section of two branches at heights of 2–3 and 5–6 m, respectively. All samples were taken from the outer part of the crown. The average sample size (±SE, *n* = 20) was 399.5 ± 67.6 (seedlings), 408.4 ± 54.4 (saplings), and 381.4 ± 54.7 (adults) leaves per tree.

Mined leaves (mean ± SE = 90.2 ± 6.4 mines per tree, range = 22–271, *n* = 60) were classified into three morphotypes. *Blotch* mines were formed by the larvae of the sawfly *Notofenusa* sp. (Hymenoptera: Tenthredinidae); *Linear* mines were ∼1 mm wide galleries that cut through the midrib and were created by a moth species (Gracillariidae); *Serpentine* mines of varied patterns were produced by two unknown lepidopterous moths. To estimate miner abundance in each canopy layer, we counted the total number of leaf miners per sample. Vertical changes in miner herbivory were assessed by scoring the percentage leaf area damaged (*n* = 30 leaves per tree) (Mazía *et al.*, 2012). The amount of leaf damage has been related to the ability of parasitoids to find hosts in forest canopies (Yamazaki, 2010).

Leaf mines were dissected under the microscope (100×) within 2 months of collection to assess parasitism rates (% parasitised mines). Miner larvae were classified as successful, parasitised, or dead by causes other than parasitism. At the time of sampling (January), successful mines were empty and had a small rounded hole (through which the larva vacates the mine to pupate in the forest floor), or were otherwise occupied by a living larva with no signs of parasitism. Living larvae were

incubated for 3 months; when no insect emerged, the larva was dissected to determine whether it had escaped parasitism. By contrast, parasitised mines contained the remains of a dead leaf miner and the pupa or larva of a parasitic wasp. Mines containing a dead miner larva but no visible parasitoid were dissected, and if no parasitoid was found, we assumed that the leaf miner had been killed by unknown causes. We found one miner larva per leaf mine and one parasitoid per mine, yet occasionally there were two mines per leaf. As we worked on a deciduous host, all mines were formed within a single season. Parasitoids were reared in the laboratory for identification; all emerged wasps belonged in the superfamily Chalcidoidea and could be identified to genus (Dr Daniel Aquino, División de Entomología, Facultad de Ciencias Naturales y Museo, La Plata, Argentina).

Leaf miner abundance was expressed as mean number of mines per unit leaf fresh mass (1 g ≈ 20 leaves) per tree ($n = 10$ trees, for each layer and site). Each leaf sample was weighed and the number of mines was divided by the total leaf mass sampled from each tree. This procedure standardised comparisons between strata differing in foliage cover and mean leaf size. The relationship between absolute miner density (no. of miners m^{-2}) and the amount of tree foliage (g dry mass m^{-2}) in each canopy layer was assessed through linear regression, including the three strata from both sites ($n = 6$). Parasitism rate (%) was calculated by dividing the number of parasitised leaf miners by the total number of miners in a sample. Patterns in miner abundance, leaf damage, parasitised miners, and parasitism rate (%) were examined using a two-way ANOVA, with forest site (PC, CH) and canopy layer (lower, middle, upper) as main effects. Because the number of parasitised miners and total parasitism rate (%) were positively correlated to leaf miner abundance across the whole dataset ($r = 0.59$, $P = 0.0001$ and $r = 0.278$, $P = 0.031$, respectively; $n = 60$ trees), analyses for parasitism included miner abundance as a covariate in type I ANCOVA models. These analyses were performed using STATISTICA software (StatSoft, Inc., 1999).

Quantitative network analysis

We counted the number of distinct leaf miner–parasitoid interactions (linkage richness) observed per canopy layer, pooling the foliage samples collected for each tree-height class. We constructed rarefaction curves assuming that linkage richness increases asymptotically with the number of observed interaction events. The null expectation was that vertical changes in linkage richness, a measure of web complexity (Bersier *et al.*, 2002; Tylianakis *et al.*, 2007), may be explained by the number of links found across canopy layers. This procedure also allowed us to assess the robustness of the networks sampled (Blüthgen, 2010). We calculated the maximum number of expected interactions (EI_{max}) for each layer using the Chao-1 estimator for asymptotic richness (Magurran, 2004). Rarefaction curves and EI_{max} were computed using the functions 'rarefy' and 'estimate' of the VEGAN package in the R programme (Oksanen *et al.*, 2010).

Quantitative networks were built for the whole miner–parasitoid community sampled in each forest site, as well as

for each separate canopy layer using the BIPARTITE package (Dormann *et al.*, 2009; R Development Core Team, 2011). We were interested in determining whether the host–parasitoid network encountered at a given height was a reduced version of the whole-community network or whether there was vertical turnover in species composition and pairwise interactions. To generate the whole-community network, we pooled over data from the three-height classes sampled within a forest. Networks for separate canopy strata were constructed by pooling over individual trees sampled within a height class. This precluded replication within sites (cf. Morris *et al.*, 2015), but allowed us to describe food webs for two independent sites based on larger samples (Paniagua *et al.*, 2009). Network links represented the number of parasitoid wasps that emerged from each miner morphotype (Lewis *et al.*, 2002; Murakami *et al.*, 2008). When a morphotype was present in a canopy layer, but could not be linked to any parasitoid, it was excluded from calculations of network metrics (Dormann *et al.*, 2009). Quantitative network graphs were produced using code developed by T. Hirao in R language (Hirao & Murakami, 2008). To visualise vertical changes in network size and topology, we depict actual insect numbers per unit leaf mass, rather than relative abundances within trophic levels (cf. Lewis *et al.*, 2002; Valladares *et al.*, 2012).

We computed six quantitative network metrics (Bersier *et al.*, 2002; Dormann *et al.*, 2009): linkage richness, the number of trophic links between different miner and parasitoid species; linkage density, the mean number of links per insect species weighted by the total number of interactions in the network; generality, the mean number of leaf miner hosts attacked by a parasitoid species; vulnerability, the mean number of parasitoids, regardless of species, attacking a given miner morphotype (both metrics were weighted by the frequency of pairwise interactions); interaction strength, defined as the mean number of interactions recorded between a given pair of miner and parasitoid species divided by the total number of links for that miner species; and interaction evenness, the Shannon diversity index based on the frequency of different pairwise trophic links divided by (ln) linkage richness. These metrics provided composite measures of network complexity, while also reflecting the strength of top-down control on leaf miners (Tylianakis *et al.*, 2007; Kaartinen & Roslin, 2011; Valladares *et al.*, 2012).

Null model analysis of network structure

To evaluate the extent to which insect abundance influenced network metrics, we compared the miner–parasitoid food web observed in each canopy layer against the equivalent randomised network generated from the original data (Vázquez *et al.*, 2005). The null hypothesis was that food web structure was generated by the number of random encounters between parasitoids and leaf miners, regardless of species identities (Vázquez *et al.*, 2009a). First, we calculated the null interaction matrix for each canopy layer by assuming that host–parasitoid links were determined solely by species abundance. The expected probability for each link was estimated as the product between

the relative abundances of the miner and parasitoid species engaged in the interaction. Then, we used a randomisation algorithm implemented with the function 'nestats' in R (after Vázquez *et al.*, 2009b) to test whether the observed network metrics for each canopy layer differed from those derived from the expected (random) interaction matrix. The algorithm reassigned the total number of interactions in the original data according to the probability matrix computed for each network, with the constraint that each species was involved in at least one interaction. The expected mean ($\pm$95% CI) value for each metric was computed on the basis of 99 randomised networks. Network metrics outside the range ($\pm$95% CI) derived from the null model suggested that factors other than insect abundance *per se* shaped miner–parasitoid food web structure (Vázquez *et al.*, 2005, 2009a).

Results

Vertical canopy structure

Tree foliage cover was lowest for seedlings ($F_{2,24} = 16.07$, $P < 0.0001$; Figure S1, Supporting Information). Sites differed in sapling and adult tree densities (site, $F_{1,24} = 6.49$, $P = 0.018$; canopy layer $\times$ site, $F_{2,24} = 3.38$, $P = 0.051$), and foliage vertical distribution (canopy layer $\times$ site $F_{2,24} = 17.21$, $P < 0.0001$). The PC forest had a more continuous vertical structure, with no difference in foliage cover and basal area between the middle and upper canopies. The CH forest had a well-developed upper canopy, but a sparse middle layer with few saplings (Figure S1, Supporting Information). The PC forest was undergoing an internal regeneration phase, with elevated sapling density and foliar cover in large treefall gaps, whereas the CH forest had an even-aged, mature overstorey.

Vertical changes in foliage quality

We found few clear-cut patterns of vertical variation in measured leaf traits across canopy strata, while trait-specific differences between forest sites were often significant (Figure S2, Supporting Information). *Nothofagus pumilio* seedlings tended to have smaller leaves, with a lower N and higher phenolic content than saplings and adult trees. On average, the CH forest trees had larger and thinner leaves, with higher water and lower phenolic contents, than the PC forest (Figure S2, Supporting Information).

Patterns of herbivory and parasitism

We collected a total of 5413 mined leaves from both forest sites, including 358 leaves with definite signs that the mining larva had been attacked by a parasitic wasp. This yielded an overall parasitism rate of 6.6% for the leaf miner guild. Mortality attributed to causes other than parasitoids accounted for 9.5% of all miners. Total leaf miner abundance (per unit leaf mass) differed between sites (PC > CH), but did not vary significantly across canopy strata within sites (Fig. 1a). This resulted from
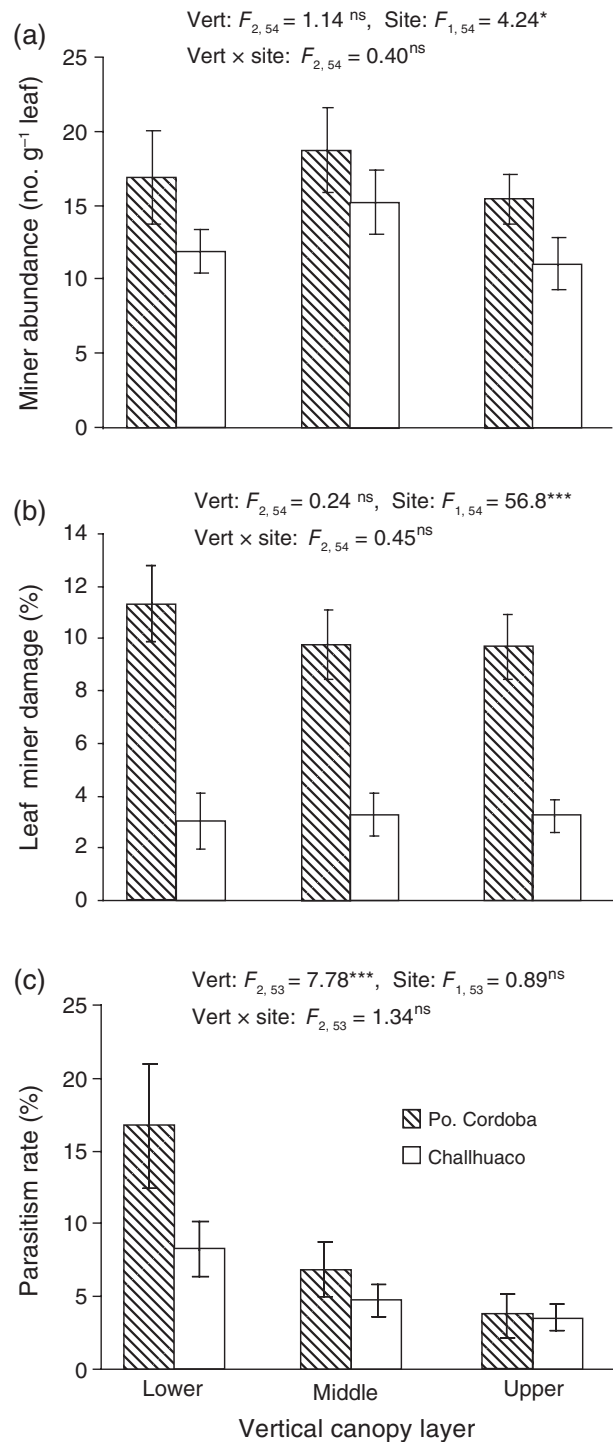

Fig. 1. Patterns of leaf miner abundance (a), leaf area damage (b), and parasitism rate (c) for three canopy layers at two *Nothofagus pumilio* forest sites (Paso Córdoba and Valle Challhuaco) in Patagonia, Argentina. Vertical canopy layers (Vert: lower, middle, upper) represented seedling, sapling, and adult trees, respectively. Bars show means $\pm$ SE ($n = 10$). Statistical results from ANOVA (a, b) and ANCOVA (c) are shown: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns$P > 0.05$.

Table 1. Quantitative network metrics of leaf miner–parasitoid food webs for three canopy layers in two *Nothofagus pumilio* forests (Paso Córdoba and Valle Challhuaco) in northern Patagonia, Argentina.

	Paso Córdoba			Valle Challhuaco		
Network metric	Lower	Middle	Upper*	Lower	Middle	Upper*
Leaf miner richness[†]	2	3	3	3	2	2
Parasitoid richness	5	6	5	6	4	2
Linkage richness	8	8	6	8	6	3
Linkage density	1.92	2.20	1.27	1.92	2.39	1.65
Quantitative generality	1.29	1.13	1.08	1.41	1.69	1.80
Interaction strength	0.24	0.14	0.13	0.19	0.065	0.045
Interaction evenness	0.62	0.65	0.41	0.87	0.95	0.92
Quantitative vulnerability	2.54	3.28	1.47	2.43	3.09	1.50

*Canopy layers sampled at 0.5–1 (lower), 2–3 (middle) and 5–6 m (upper) height.
†The number of mine morphotypes (blotch, linear, serpentine) that were linked to at least one parasitoid in the network. However, all three morphotypes were found throughout the canopy in both forests.
Canopy layers corresponded to different tree-height classes: seedlings (lower, 0.5–2 m), saplings (middle, 3–5 m), and adults (upper, 8–20 m). Metrics were computed from foliage samples ($n = 10$ trees) pooled within canopy strata.

mean leaf miner densities (no. miners m^{-2}) per stratum being directly proportional to the amount of foliage (g leaf m^{-2}) in each canopy layer ($r^2 = 0.88$, $n = 6$, *slope* = 14 mines g^{-1} leaf). Leaf area damage by mining insects was also greater in PC than in CH, but did not vary across canopy layers (Fig. 1b).

The number of parasitised miners declined from the lower, through the middle to the upper canopy layers ($F_{2,53} = 5.98$, $P = 0.0046$; site × layer, $F_{2,53} = 1.69$, $P = 0.19$), and did not differ between sites after adjusting for miner abundance ($F_{1,53} = 0.96$, $P = 0.33$; covariate $F_{1,53} = 36.7$, $P < 0.0001$). Moreover, parasitism rates (%) decreased with canopy height after controlling for miner abundance (covariate, $F_{1,53} = 6.06$, $P = 0.017$; Fig. 1c). The intensity of parasitism was significantly greater for leaf miners feeding on understorey seedlings than for those on saplings or adult trees (Tukey test $P < 0.05$; Fig. 1c).

Vertical stratification of host–parasitoid food webs

The whole-community web in both forests comprised three miner morphotypes and the same six parasitoid species. Yet dominant leaf miner and parasitoid taxa differed between sites (Figure S3, Supporting Information). The blotch-type sawfly produced 93% of all mines in the PC forest, whereas linear-shaped mines accounted for 70% of mined leaves in the CH forest. The dominant miner in each forest was attacked by most parasitoid species (PC, blotch miner, six spp; CH, linear miner, five spp); the least abundant, serpentine mines supported the same three parasitoids in both forests. The parasitoid assemblage comprised one Pteromalidae specialised on the blotch leaf miner, and five species of polyphagous Eulophidae, of which the most common was a *Neochrysocharis* sp. 1 parasitising all three miner morphotypes. Both community networks had a moderate linkage richness (9–11), relative to the total number of possible links (18). The degree of generality (1.3–1.6) indicated that most parasitoids attacked one or two leaf miners at a given site. Community networks differed in that interaction evenness was higher in the CH forest (Figure S3, Supporting Information).

Linkage richness decreased from the lower to the upper strata, especially in the CH forest, where it paralleled an upward decrease in parasitoid richness (Table 1, Fig. 2). The specialist Pteromalid feeding on the blotch miner was common in the CH understorey, but was missing from higher canopy webs. Also, two Eulophidae parasitising the dominant linear miners in the CH understorey were not found on adult trees (Fig. 2: right panels). Rarefaction analysis indicated that the loss of trophic links with canopy height tracked an upward decline in the number of interactions being recorded (Fig. 3). The numbers of links (per unit leaf mass) in the upper canopy were 17% (CH) and 47% (PC) of that recorded in the understorey. Overall, few novel links were found as the number of interactions increased within a canopy layer (Fig. 3). Rarefaction curves approached the predicted linkage richness within Chao-1 confidence limits, suggesting that our sampling effort was adequate for both sites. Notice that confidence limits were rather wide in the PC middle and upper strata (Fig. 3) due to increased numbers of rare (weak) trophic links (see Fig. 2).

Food web topology changed with canopy height, even though dominant leaf miner and parasitoid taxa did not vary across strata (Fig. 2). Upper canopy food webs were depauperate versions of lower canopy ones. There was no vertical turnover among the three miner morphotypes, and no parasitoid species was exclusive to the upper canopy. Only the least common parasitoid (*Neochrysocharis* sp. 5) was missing from the understorey in the PC forest (Fig. 2). Network metrics depicted this vertical variation in food web topology. Mean interaction strength, linkage density, and host vulnerability were all at their lowest in the upper canopy of both forests, although the latter two metrics peaked in the middle layer (Table 1). Quantitative generality and interaction evenness also decreased towards the upper canopy in the PC forest, but followed the opposite trend in the CH forest, where only two parasitoids occurred in the overstorey (Table 1, Fig. 2). Still, vertical differences in generality were fairly small in both forests.

Our null model analyses revealed that food web metrics were often lower than expected by chance alone (Fig. 4). Half the
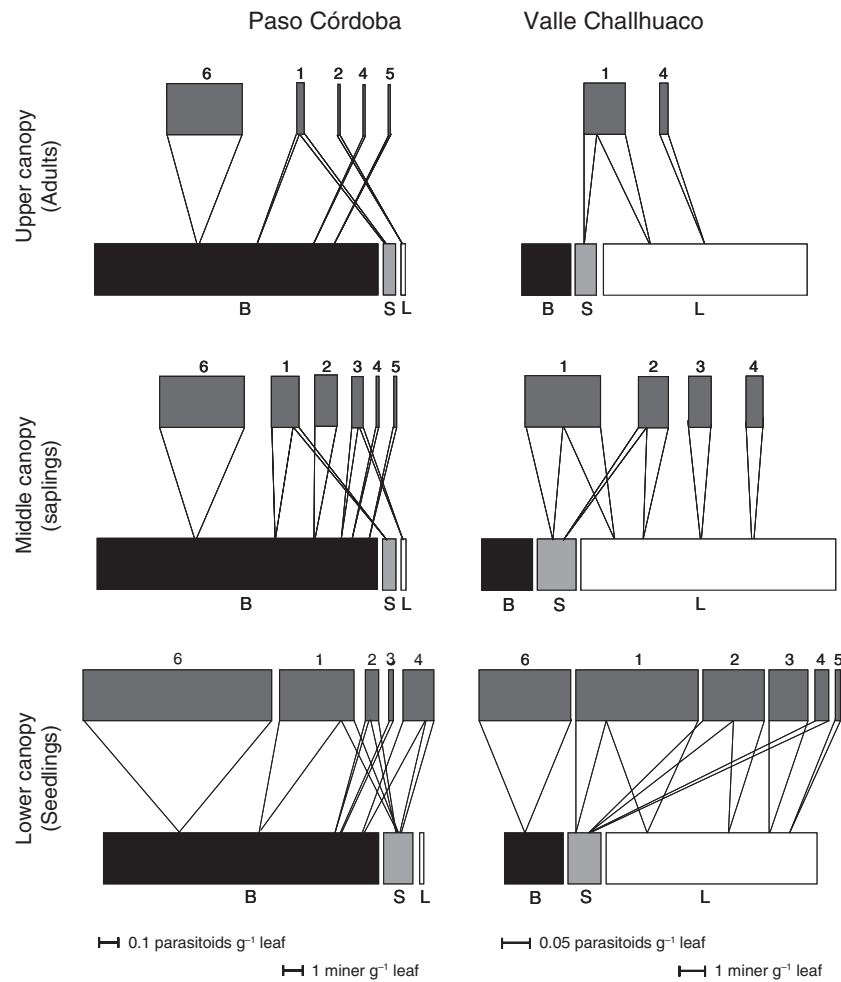
Fig. 2. Leaf miner–parasitoid networks for three canopy layers at two *Nothofagus pumilio* forest sites (Paso Córdoba and Valle Challhuaco) in Patagonia, Argentina. Rectangles show species abundance (no. insects g^{-1} leaf mass) in each trophic level; miner morphotypes: blotch (B), serpentine (S) and linear (L); parasitoid species (1–6). The width of each link reflects the proportion of a given parasitoid attacking a host morphotype. Scale bars are given for each forest site. Parasitoid taxa (Hymenoptera, Chalcidoideae) comprised five Eulophidae: *Neochrysocharis* spp. (1, 2, 4, 5) and *Elachertus* sp. (3); and one unidentified Pteromalidae (sp. 6).

network metrics (15/30) fell below the lower confidence limit of the null expectation, and this pattern was particularly strong in the upper canopy of both sites (8/10 metrics; Fig. 4). If the three miner morphotypes had been attacked by these parasitoid species at the same overall intensity, but in a random fashion, we would have seen more complex networks, with greater levels of linkage richness, linkage density, generality, and vulnerability. The exception was interaction evenness, which was greater than expected in the middle and upper layers of the CH forest (Fig. 4).

Discussion

We set out to examine how forest vertical structure influenced food webs of leaf miners and their parasitoids. We found that food web complexity decreased from lower to upper canopy strata, although saplings and adult trees provided more foliar resources and habitat for insects. This vertical pattern

corresponded with a marked decline in overall parasitism rates with canopy height. Moreover, food web metrics were often lower than expected for randomised networks only structured by species' relative abundances, particularly in the upper canopy. Thus, poor trait matching between leaf miners and resident parasitoids, as well as species abundance, appeared to influence food web assembly in these Patagonian forests (Jordano *et al.*, 2003; Vázquez *et al.*, 2009a).

We initially expected that leaf miner abundance per unit leaf mass and parasitism rates would increase from lower to upper canopy strata, through a 'bottom up' effect from foliage cover on habitat and resources available to insects (Price, 2002). However, we found that neither miner abundance nor leaf damage by mining insects differed across strata (Fig. 1a,b). This pattern emerged as absolute miner densities (no. m^{-2}) tracked variation in the amount of foliage provided by *N. pumilio* at different canopy heights, with foliage biomass being lowest in the understorey (Figure S1, Supporting Information). Results
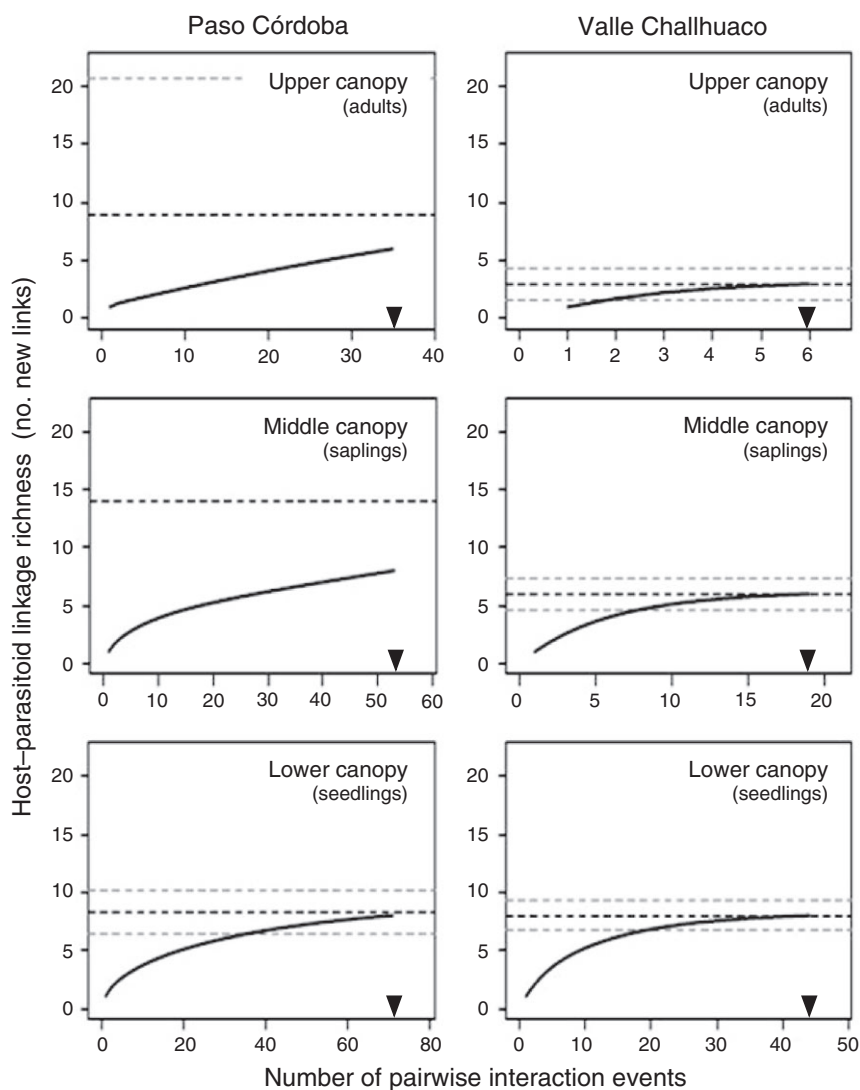
Fig. 3. Rarefaction curves for the number of leaf miner–parasitoid links (linkage richness) encountered at three canopy layers (lower, middle, upper) in two *Nothofagus pumilio* forests (Paso Córdoba and Valle Challhuaco) in Patagonia, Argentina. Triangles show the observed number of interactions for each canopy layer and forest site. Dashed lines (black) indicate the maximum number of links expected for each canopy layer (±1 SE, grey lines). The *x*-axis scale varies across panels, reflecting the actual number of interaction events encountered at different canopy heights.

also suggested that middle and upper canopy layers provided better quality foliage, based on higher N and lower phenolic contents (Figure S2, Supporting Information), but this trend was not mirrored by changes in miner abundance (g^{-1} leaf) or herbivory levels. Still, how mining insects responded to upper canopy conditions beyond the maximum sampling height (6 m) is unknown. Thus, within the vertical scope of this study, leaf miners appeared to exploit foliar resources in direct proportion to their availability at different canopy strata (cf. Brown *et al.*, 1997).

Parasitism rates decreased towards the upper canopy (Fig. 1c), where absolute miner densities (no. m^{-2}) were greater than in the understorey. This pattern held for both forests despite site-level differences in leaf miner herbivory (Fig. 1a,b) and canopy structure (Figure S1, Supporting Information). Our results suggest an

inverse density-dependent response of parasitoids to leaf miner density across strata. Equivalent limitations to exploit abundant prey have been noted before for hymenopterous parasitoids (Heads & Lawton, 1983; Godfray, 1994), and point to factors other than resource supply as possible causes for the lowered parasitism found in middle and upper forest strata. First, parasitoids may show a reduced functional response to increased mine densities due to constraints imposed by the number of eggs carried by female wasps (Heimpel *et al.*, 1996, 1998). Also, the greater structural complexity of adult tree crowns may contribute to reduce parasitoids' foraging efficiency and attack rates (Godfray, 1994; Yamazaki, 2010). Secondly, parasitoids may exhibit a reduced aggregative response at the scale of study (Heads & Lawton, 1983), perhaps due to limited dispersal from overwintering sites in the forest floor. Indeed, low
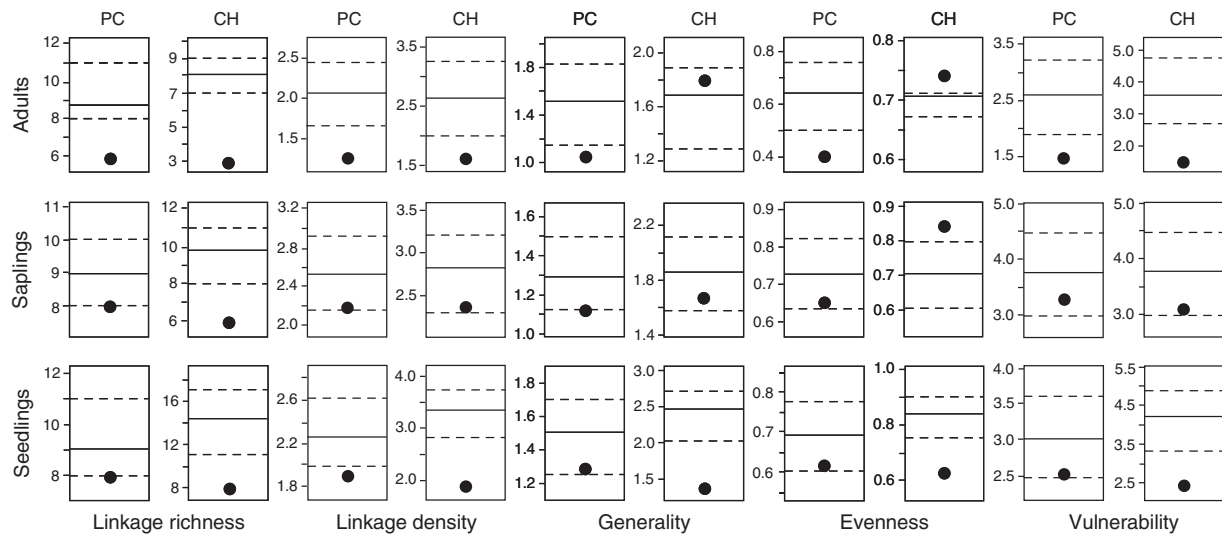
Fig. 4. Null model analysis of leaf miner–parasitoid network metrics showing values obtained from field data (black dots) and expected (solid lines) from a randomisation procedure (with ±95% CI, dashed lines). The analysis was performed separately for networks in three height classes (seedlings, saplings, adults) in two *Nothofagus pumilio* forests (PC, Paso Córdoba; CH, Valle Challhuaco). A black dot falling inside the dashed lines indicates that the observed metric did not differ from that expected by chance, as predicted from relative species abundances in the network.

dispersal capacity often leads to spatial aggregation of parasitoids irrespective of host densities (Godfray, 1994; Briggs & Latto, 2000). Lastly, vertical changes in canopy microclimate could affect the intensity of miner–parasitoid interactions (Basset *et al.*, 2003; Ulyshen, 2011). For instance, increased wind speed in the upper canopy may reduce parasitoid searching time and oviposition rates (Weisser *et al.*, 1997).

The upward decline in parasitism rates influenced the vertical pattern of food web structure. As the frequency of pairwise interactions decreased with canopy height, so did parasitoid and/or linkage richness (Table 1, Figs 2 and 3). This resulted in less complex food webs on adult trees, with overstorey networks being impoverished subsets of understorey ones. Within-habitat heterogeneity in food web structure was probably driven by parasitoid species dynamics, as the host tree and leaf miner species were the same in all three forest strata. Likewise, Paniagua *et al.* (2009) reported a vertical decrease in complexity of species-rich webs of gall-making insects and their parasitoids in a tropical forest. Yet they found substantial species turnover between undestorey and canopy assemblages (Paniagua *et al.*, 2009), reflecting the highly diverse structure of tropical plant communities (Basset *et al.*, 2003; Lewinsohn & Roslin, 2008). Basset *et al.* (2003) posited that stratification of arthropod assemblages may depend on the vertical discontinuity of canopy habitats, which would be greater in mixed or tropical rainforests than in monotypic or temperate forests. The similarity between our results and those of Paniagua *et al.* (2009) contradicts this hypothesis, while pointing to the need for more comparative studies on vertical stratification of host–parasitoid networks across forest types.

Quantitative network metrics were generally consistent in showing that the loss of parasitism led to simplified food webs in the upper canopy (Table 1, Fig. 2). Linkage density and mean interaction strength were at their lowest in adult tree networks.

Both the diversity and frequency of trophic connections peaked in the middle canopy, but declined abruptly in the overstorey. The vertical decrease in linkage density could be attributed to the loss of certain links (linkage richness) as well as to the reduction of mean interaction strength, two elements that are tightly related in bipartite networks (Dormann *et al.*, 2009; see Fig. 3). As a consequence, the weighted number of parasitoid species feeding on each host morphotype (quantitative vulnerability) was also lowest on adult trees. These results imply that the overall intensity of parasitoid control on leaf miners may be compromised in the upper canopy (Ribeiro & Basset, 2007; Paniagua *et al.*, 2009). On the other hand, the greater web complexity found in the middle storey (Table 1, Fig. 2) could reflect a trade-off between parasitoid dispersal from the litter layer (Paniagua *et al.*, 2009) and harsher abiotic conditions in the upper canopy (Basset *et al.*, 2003; Ozanne *et al.*, 2003). It must be acknowledged, however, that our sampling did not include the upper section of adult tree crowns. From present findings, we would expect a further reduction in the complexity of host–parasitoid networks supported by tree tops (see also Brown *et al.*, 1997; Basset *et al.*, 2003; Paniagua *et al.*, 2009).

Vertical trends in network attributes were broadly consistent between forests, despite shifts in dominant miner morphotype and parasitoid species (Table 1, Fig. 2). Site-specific differences in leaf miner dominance could help to explain food web patterns, such as the lower evenness of PC forest webs, which reflected the prevalence of a specialist Pteromalid attacking the blotch miner (see Figure S3, Supporting Information). A shift in dominant leaf miner might also drive vertical changes in parasitoid assemblages. Whereas the blotch miner was attacked by most parasitoid species throughout the PC forest canopy, the linear miner lost several parasitoids in the upper CH forest canopy (Fig. 2). Site-level differences in canopy physiognomy also appeared to influence food web patterns (Basset *et al.*,

2003). We found that the upward loss of network complexity was smoother in the PC forest, which had a more continuous canopy with a dense midstorey of saplings, compared with the CH forest dominated by mature trees (Figure S1, Supporting Information). Thus, vertical loss of linkage density resulted from fewer interactions per link in PC, but reflected the loss of certain trophic links in CH (see Table 1, Fig. 2). Our findings support the idea that vertical canopy structure may affect not only insect species distribution (Basset *et al.*, 2003; Ulyshen, 2011), but also food web properties within forest habitats.

We found that observed network metrics in both forests often fell close to, or outside, the confidence limits derived from null models (Fig. 4). This finding indicates that food web structure may not be simply explained by random host–parasitoid encounters generated by species' relative abundances (Vázquez *et al.*, 2005). Instead, the food webs studied here would be shaped by a mismatch between leaf miner and parasitoid functional traits as well as by species abundance (Jordano *et al.*, 2003; Vázquez *et al.*, 2009a). This pattern was particularly true for the upper forest canopies, where overall parasitism rates were the lowest (Figs 1c and 4), which suggests that 'neutral' interactions (*sensu* Vázquez *et al.*, 2009a) would have played a greater relative role in structuring host–parasitoid webs in the lower and middle canopy strata.

There are several reasons why food web complexity may be lower than expected from random host–parasitoid interactions. First, it is possible that 'missing links' were generated by sampling effects, leading to the under-representation of weak (or rare) interactions (Vázquez *et al.*, 2009a,b; Olesen *et al.*, 2010). However, sample sizes and number of dissected mines did not substantially differ among strata (see Methods), which should lower the chances that reduced network complexity in the upper canopy was caused by sampling limitations (Blüthgen, 2010). Second, 'forbidden links' among parasitoid and miner species may arise (Jordano *et al.*, 2003; Vázquez *et al.*, 2009a) due to morphological (e.g. body size) or behavioural mismatches that prevent parasitoids from attacking leaf miners as encountered (Godfray, 1994; Petchey *et al.*, 2008; Lepänen *et al.*, 2013). This could be the case for the Pteromalid sp. 6, which had the largest pupae and was only seen feeding on the sawfly blotch miner, despite being relatively common in both forests (see Fig. 2). Third, food web complexity may be affected by colonisation rates (Pillai *et al.*, 2011; Fahimipour & Hein, 2014), as shown for leaf miner–parasitoid networks in fragmented habitats (Murakami *et al.*, 2008). It is possible that limited parasitoid dispersal from pupation sites in the litter layer had not only restricted total parasitism (Fig. 1c), but also linkage strength and richness in the upper canopy (Figs 3 and 4). We suggest that the parasitoids' differential dispersal ability and adaptability to upper canopy conditions may act to reinforce the role of species identity in shaping food web topology across forest strata (Paniagua *et al.*, 2009; Fahimipour & Hein, 2014). Fourth, the fact that network metrics across the whole system could be lower than predicted by our null models highlights the potential for biogeographic or phylogenetic constraints to affect the phenotypic traits present in the regional species pool (Vázquez *et al.*, 2009a; Pillai *et al.*, 2011; Lepänen *et al.*, 2013).

Our study provides the first quantitative account of the structure of insect food webs in a well-preserved South American temperate forest. When compared with other host–parasitoid webs around the world (Morris *et al.*, 2014), our forest networks stand out for their small size and relative simplicity (Figure S3, Supporting Information). This is not surprising given the monotypic nature of *N. pumilio* forests, which would provide fewer niche opportunities for herbivorous insects (Lewinsohn & Roslin, 2008; cf. Kaartinen & Roslin, 2011), and the diversification of parasitoid assemblages (Lepänen *et al.*, 2013). Further, suboptimal temperature regimes (Deutsch *et al.*, 2008), coupled with the biogeographic isolation of Patagonian forests (Aizen & Ezcurra, 2008), may also contribute to limit canopy insect diversity and food web complexity in the study system.

In conclusion, we found strong vertical structuring of leaf miner–parasitoid food webs harboured by *N. pumilio* trees in these Patagonian forests. Parasitism rates declined with tree height, leading to the loss of interaction diversity and food web complexity in the upper canopy. Our findings suggest that the parasitoids' limited functional response to host abundance and dispersal within the forest canopy might contribute to the observed vertical stratification of host–parasitoid food webs. Nevertheless, food web attributes were not explained solely by species abundances, but could also be influenced by phenotypic mismatches between miner morphotypes and parasitoid species (Vázquez *et al.*, 2009a; Blüthgen, 2010). Our study suggests that the functional diversity of these host–parasitoid assemblages depends on lower canopy strata, while the forest overstorey supports the bulk of leaf miner hosts. Thus, human activities altering the understorey may have unexpected consequences on canopy insect communities and the trophic control of tree folivory rates.

Acknowledgements

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12332

   Figure S1. Vertical forest structure.
   Figure S2. Tree foliage characteristics.
   Figure S3. Whole community networks.

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