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Saproxylic beetle communities above, within and below tree canopies: a methodological breakthrough highlights the habitat specificity of the canopy-aerosphere interface

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Abstract

Forest biodiversity is strongly vertically stratified, yet the aerial space immediately above the canopy remains largely overlooked. We investigated whether the canopy-aerosphere interface (CAI) constitutes a distinct habitat for flying saproxylic beetles. In addition, we developed the Canopix® system to enable standardized sampling across the entire vertical forest gradient. We sampled mature oak forests in central France over two consecutive years, compared beetle assemblages in the CAI with those within and below the canopy, and assessed temporal variations, forest dieback and tree position effects. We collected 38,183 individuals representing 514 species.

Assemblages in the CAI consistently differed in taxonomic composition from those in the lower strata, with dissimilarity driven primarily by species turnover rather than nestedness. Local species richness and functional richness were broadly comparable among strata, while the CAI harboured more exclusive and conifer-specific species. Despite pronounced seasonal turnover, CAI distinctiveness persisted throughout both activity periods and was comparatively insensitive to forest dieback and proximity to the vertical forest edge, suggesting that the CIA may provide an aerial movement corridor, among other ecological functions. Our results identify the CAI as a distinct and previously overlooked component of forest biodiversity, with implications for insect monitoring and conservation.

1. Introduction

Forests are vertically stratified ecosystems in which abiotic conditions change markedly from the understory to the outermost tree crown (Ozanne et al., 2003; Parker and Brown, 2000). At their upper boundary, the canopy meets the free atmosphere, forming the transition zone first described as the “canopy ecotone” (Hallé and Blanc, 1990) and more recently referred to as the “canopy-aerosphere interface” (CAI; Cunningham-Minnick et al., 2023). This compartment constitutes an abrupt ecological transition between the structurally complex canopy, characterized by abundant woody resources, numerous tree-related microhabitats and relatively sheltered conditions (Lowman and Rinker, 2004), and the atmospheric boundary layer, where vegetation is virtually absent except for occasional dead branches protruding above the canopy in declining forests (Drénou et al., 2011). In this respect, the CAI can be viewed as a horizontal forest edge, marking the abrupt transition between forest habitat and the surrounding open atmosphere. Similar ecological contrasts occur along the vertical forest edge at the boundary between a forest stand and adjacent open habitat. Both interfaces are characterized by increased exposure to incoming solar radiation and higher evaporative demand, which can elevate their temperature (Vinod et al., 2023), as well as stronger winds and more turbulent airflows than in strata situated deeper within the forest, either lower in the canopy or farther from the stand edge (Brunet, 2020). Along with the moisture released by canopy evapotranspiration (Monteith and Unsworth, 2013), these factors create distinctive microclimatic environments that differ markedly from conditions prevailing in the forest interior (De Frenne et al., 2019). Altogether, these contrasts in habitat structure, in resource and tree-associated microhabitat availability, and in abiotic and aerodynamic conditions suggest that the CAI should be regarded as a distinct ecological habitat compartment rather than simply

as the uppermost limit of the forest canopy. When these two interfaces occur in close proximity, as in trees located near the stand edge, their effects may interact (Didham and Ewers, 2014). The proximity of the vertical forest edge may therefore generate a double edge effect, potentially modifying the environmental gradient and ecological conditions associated with the CAI. Furthermore, forest dieback can alter canopy structure (Salle et al., 2021) and may consequently modify the vertical edge effect associated with the CAI itself. Together, the proximity of trees to a vertical forest edge and forest dieback may therefore influence the ecological functioning and distinctiveness of the CAI.

To address these questions, we focused on saproxylic beetles, a group of species that depend on dead or decaying wood and associated tree-related microhabitats (Speight, 1989). Saproxylic beetles are widely used as model organisms in forest ecology due to their high diversity, close association with forest structural complexity and to the key ecological functions they perform, particularly wood decomposition and nutrient cycling (Stokland et al., 2012). Although fundamentally linked to woody substrates, many saproxylic species are highly mobile and rely on flight for dispersal and mate-finding (Ulyshen, 2011).

The vertical distribution of saproxylic species within the forest has received increasing attention over the past decade, with most studies demonstrating that saproxylic beetle assemblages differ markedly among forest strata (Balvay et al., 2026; Bouget et al., 2011; Meng et al., 2022). However, these studies have focused on the understory and canopy strata, leaving the CAI unexplored. To our knowledge, no study to date has characterised the saproxylic beetle communities inhabiting this compartment in temperate forests.

This knowledge gap largely reflects methodological constraints. Early studies of forest canopies relied on ground-based observations or destructive tree felling (Barker and Pinard, 2001). Later technical developments enabled direct canopy access through permanent infrastructures such as canopy towers (Haddow and Corbet, 1961) and cranes (Basset et al., 2004), or more flexible approaches including single-rope climbing (Perry, 1978), rope bridges (Muul and Liat, 1970) and canopy rafts (Hallé et al., 2000). Insecticide knockdown also improved canopy arthropod inventories without direct access (Erwin, 1982). However, all the above approaches involve trade-offs. Permanent infrastructures are expensive and restricted to a limited number of study sites, while climbing is labour-intensive making passive traps impossible to check over extended periods. Likewise, insecticide knockdown provides only an instantaneous, unstratified sample and is destructive to all the arthropods inside the vertical column. Thus, none is suited to standardized, long-term sampling across large numbers of trees, either in the CAI or, for that matter, in the rest of the vertical gradient. Recently, Cunningham-Minnick et al. (2022) developed the first system specifically designed to access the CAI. However, to maximise portability and minimise costs, the system was designed to support a single lightweight sampling device, precluding the simultaneous deployment of multiple traps along the vertical forest gradient.

To overcome these limitations, we developed a novel sampling device, called the Canopix®, designed to simultaneously access the CAI and sample the entire vertical gradient of a tree. Because of its

lightweight design, the Canopix® can be transported and installed in remote or difficult-to-access forest sites, providing a practical solution for standardized, long-term, arthropod sampling.

Thanks to this methodological breakthrough, we investigated whether the CAI constitutes a distinct habitat for saproxylic beetles. Specifically, we tested whether (i) flying saproxylic beetle assemblages at the CAI, above the canopy, differ from those occurring within and below the canopy, (ii) whether any such distinctiveness varies over the course of the insect trapping season, from spring to fall, (iii) whether this possible distinctiveness is shaped by forest dieback, and (iv) whether it is influenced by the proximity of trees to the vertical forest edge.

2. Material and methods

2.1. Study area and sampling methods

This study aimed to assess the distinctiveness of the flying saproxylic beetle assemblages sampled in the CAI compared to the ones sampled within and below the canopy. The study sites were chosen in even-aged, managed high-forest stands dominated by mature *Quercus robur* and *Q. petraea* in four public forests in central France (Fig. 1). To investigate the effects of forest dieback and proximity to the vertical forest edge, two field campaigns were conducted over two consecutive years.

2.1.1 Healthy *vs*. declining forest plots

In 2024, we surveyed twenty plots spanning a dieback gradient from healthy to declining located in the Vierzon and Tronçais State Forests (Fig. 1). Plot health was quantified with a dieback index integrating (i) branch mortality and foliage loss of dominant oak trees and (ii) the proportion of dead oaks within a 30-m-radius circular plot centred on each sampled tree. The plots and the dieback index are described in Balvay et al. (2026). To evaluate the effect of forest dieback on canopy assemblage distinctiveness, we selected the six plots with the lowest dieback index (hereafter "healthy" plots) and the six plots with the highest index (hereafter "declining" plots). The remaining eight plots, with intermediate dieback, were included in the overall description of beetle assemblages but excluded from the analyses focusing on the effect of dieback on beetle assemblage distinctiveness in the CAI.

2.1.2 Proximity to the vertical forest edge

In 2025, we selected ten forest islands located in the Vierzon, Saint-Palais, and Orléans State Forests (Fig. 1; size and characteristics in Table S1). We defined a forest island as an isolated forest patch of mature forest surrounded by regeneration stands whose canopy height did not exceed a third of the height of the focal stand. For each island, we sampled one oak in its centre and one on its southern edge. This configuration created a clear contrast between forest-interior and forest-edge habitats, allowing us to investigate whether proximity to the vertical forest edge influences the vertical stratification of flying beetle assemblages.

2.1.2 Sampling in the canopy-aerosphere interface and along the vertical gradient with the Canopix®

Each sampled oak (*Quercus robur* or *Q. petraea*) was equipped with a Canopix® (Fig. 2; Fig.S2). The system consists of two fiberglass tubes assembled into a 9-m pole, topped with an aluminium gallows bracket. Two tree climbers install the structure in the tree crown and secure it at three anchor points. The upper and lower pole ends are stabilized by three to four guy lines attached in opposing directions to oak branches ≥ 5 cm in diameter. A third anchor point is a belt securing the pole at mid-height to the main trunk (≥ 8 cm diameter). The aluminium gallows bracket provides a suspension point up to 3.5 m above the uppermost branch, allowing traps to be suspended continuously from the CAI to ground level, thus covering the entire vertical profile. The traps are suspended from the aluminium gallows bracket and lowered through a narrow passageway cleared through the foliage by selective pruning before installation, ensuring unobstructed sliding (Fig. S2).

The Canopix® costs approximately €300 in materials (2024 prices). Its lightweight design (around 20 kg) facilitates transport and installation in remote field sites, and the structure can be deployed in half a day by experienced climbers. Up to twenty trees per season could be sampled thanks to the system.

We sampled flying beetles with black multi-funnel Lindgren traps without attractants and a grid to prevent bats from being accidentally trapped. The collection cups contained a 50% monopropylene glycol solution diluted with water and a small amount of odourless detergent and were emptied monthly between April and September 2024 and March and August 2025 (Table S3). In 2024, the traps were positioned in four vertical strata: in the CAI above the canopy (AC), in the upper canopy (UC; upper crown half), in the lower canopy (LC; lower crown half), and below the canopy (BC), beneath the crown (Fig. 2). In 2025, the design was simplified and we used a single mid-canopy trap (MC; the height for all traps in Table S4). We considered relative height more biologically relevant for defining strata than absolute height (McCaig et al., 2020).

A group of experienced taxonomists later identified the collected specimens to the species level with standard identification keys and reference collections.

2.2. Data analysis

All data analyses were conducted with the *R* software 4.5.0 (R Core Team, 2025) (references of the packages in Table S5). We applied the same analytical framework to the following datasets and objectives: (i) pooled yearly catches of the 2024 and 2025 experiments to assess whether the CAI constitutes a distinct habitat compared to the strata situated within and below the canopy; (ii) monthly catches from the 2024 experiment to evaluate seasonal variations in this possible distinctiveness; (iii) total year catch data from the 2024 experiment comparing healthy and declining plots to assess the effect of forest dieback on possible CAI assemblage distinctiveness; and (iv) total year catch data from the 2025 experiment comparing trees located at the forest island edge and those in the centre of the

forest island to assess the effect of proximity to a vertical forest edge on possible CAI assemblage distinctiveness.

2.2.1 Community composition

We first assessed the distinctiveness of the CAI in terms of taxonomic composition through non-metric multidimensional scaling (NMDS) on the dissimilarity matrices and by testing for differences with permutational multivariate analyses of variance, PERMANOVA (*vegan R* package's *metaMDS()*, *vegdist()* and *adonis2()* functions). We then performed pairwise comparisons between strata with permutation tests (9,999 permutations restricted within forests; *pairwiseAdonis R* package's *pairwise.adonis2()* function), and calculated the corresponding mean dissimilarity values. We then partitioned these dissimilarities (nestedness versus turnover; *betapart R* package's *beta.pair()* function). These analyses were run on the full species matrices as well as on reduced matrices excluding singletons and doubletons to test for the influence of rare species. Each matrix was analysed with Bray-Curtis and Jaccard dissimilarities to assess the role species abundance played in CAI distinctiveness.

Finally, we quantified the beta diversity among strata with multivariate dispersion (*vegan R* package's *betadisper()* function), whereas we assessed Gamma diversity with sample-size-based rarefaction and extrapolation curves of species richness (Hill number q = 0; *iNEXT R* package's *iNEXT()* function).

2.2.2 Community metrics

To further characterize the origin of the any CAI distinctiveness, we categorized the sampled species into functional guilds based on Bouget et al. (2019; Table 1). Species known to be strictly associated to conifers were defined as "tourist" species since we sampled only in oak trees in oak-dominated forest areas. Community weighted means (CWM) were calculated for three functional traits (shade tolerance, preferred substrate diameter and size).Functional richness (FRic; (Villéger et al., 2008) was computed based on all available species traits (Table S6; *FD R* package's *dbFD()* function).

Table 1
List of all calculated entomological metrics. ab: abundance; sr: species richness; CWM: Community Weighted Mean

Group of metrics	Abbreviation	Calculated metric types	Ecological group or trait
Taxonomic diversity	colsx	ab, sr	Saproxylic beetles
	colsxrar	ab, sr	Rare saproxylic beetles (conservation index 3 or 4)
	colsxcom	ab, sr	Common saproxylic beetles (conservation index 1 or 2)
Tourist species	colsxrsx	ab, sr	Species associated with conifers only
Multitrait functional diversity	all functional traits	FRic	Functional richness
Trophic larval guilds	pho	ab, sr	Nest-dweller
	myc	ab, sr	Mycophagous
	xyl	ab, sr	Xylophagous
	sxy	ab, sr	Saproxylophagous
	zoo	ab, sr	Predators and parasitoids
Adult guilds	flo	ab, sr	Floricolous
Ecological niche traits	canop	CWM	Shade tolerance
	diam	CWM	Prefered saproxylic substrate diameter class
	size	CWM	Size of the adult

We then used generalized linear mixed models (GLMM; *glmmTMB R* package's *glmmTMB()* function) to compare the distribution of all the response variables between the CAI and the strata situated within and below the canopy. The modelling procedure, error distributions and random-effects structure followed those described in Balvay et al. (2026).

2.2.3 Defining abundant species

To investigate whether species found to be associated with the CAI retained the same association across seasons, healthy and declining plots, and edge and interior trees, we first defined the abundant species as those represented by more than 15 individuals in the yearly catch datasets. For each abundant species and dataset, the stratum with the highest cumulative abundance was considered its preferential stratum. Species reaching identical maximum cumulative abundance in two or more strata were not assigned to any preferential stratum.

3. Results

3.1. The Canopix® provides efficient access to the whole vertical gradient of forest canopies

We deployed our novel sampling system continuously from April to October 2024 and from March 2025 to May 2026. Throughout both sampling campaigns, the structures were subjected to a wide range of weather conditions, including maximum wind speeds of 87 km per hour in 2024 and 100 km per hour in 2026 (Fig. S7). Only two of the twenty Canopix® structures failed during the first year, both because of improper installation or design flaws, whereas no structural failures occurred during the second year.

Of the 400 samples scheduled in 2024, 55 (13.8%) were lost, mostly (36%) due to traps entangled in branches or collection cups coming loose after striking branches during deployment or retrieval. Trap malfunctions accounted for another 27% of the lost samples, whereas Canopix®-related issues ranked at third accounting for 21% of the lost samples (mast breakage following incorrect installation or, in one case, rupture of the draw rope during trap retrieval). Following improvements to the installation protocol including, in particular, widening the pruned out passageway, total sample loss was reduced to only seven out of 300 (2.3%) in the second year. Two resulted from collection cups falling off, most likely after hitting branches, and two from an excessive accumulation of plant debris that rendered the traps ineffective.

We observed only one interaction between wildlife and our sampling system during the study: a blue tit (*Cyanistes caeruleus*) built a nest inside a trap positioned in the CAI during the 2025 sampling season. No damage to the trees was observed at the attachment points.

Overall, the Canopix® system enabled us to collect 19,903 saproxylic beetles belonging to 375 species, 239 genera and 50 families in 2024; and 18,280 individuals belonging to 393 species, 264 genera and 53 families in 2025 (complete species list in Table S8). For both years, one third of the recorded species were represented by only one or two individuals.

3.2. Taxonomic and functional differentiation of the canopy-aerosphere interface

3.2.1 Community composition

In both 2024 and 2025, beetle assemblages sampled at the CAI differed markedly in taxonomic composition from those collected within and below the canopy (Fig. 3). This pattern was consistent regardless of the analytical strategy adopted (Table S9) and was supported by the PERMANOVA and pairwise comparisons, confirming that the apparent separation observed in the NMDS ordinations was statistically significant (Table S10).

The CAI also exhibited greater compositional dissimilarity from the other strata than the average dissimilarity among the remaining strata. Mean dissimilarity between the CAI and the other strata exceeded the average inter-stratum dissimilarity by 2.5% in 2024 and by 13.7% in 2025 (Table 2; detailed values in Table S10).

Table 2
Mean dissimilarity values in different contexts between the CAI (AC) and the other strata (UC, LC and BC in 2024 and MC and BC in 2025) and among the other strata

		Mean dissimilarity value		
		AC vs. Other strata	Other strata	% of difference
Yearly catch	2024	0.714	0.697	+ 2.5
	2025	0.765	0.673	+ 13.7
Sampling event	April	0.730	0.728	+ 0.2
	May	0.765	0.731	+ 4.6
	June	0.804	0.800	+ 0.5
	Jul.Aug.	0.774	0.787	− 1.6
Health status	Healthy	0.718	0.710	+ 1.1
	Declining	0.708	0.657	+ 7.8
Position in the stand	Centre	0.773	0.683	+ 13.2
	Edge	0.760	0.645	+ 17.6

Partitioning Sørensen dissimilarity revealed that these differences were driven primarily by species turnover rather than nestedness. In 2024, the nestedness component accounted for only 13% of the total dissimilarity between the CAI and the strata situated within canopy, and for 8.5% of the dissimilarity between CAI and the BC stratum. In 2025, nestedness accounted respectively for 6.8% and 12% (Table S11).

Furthermore, within-stratum β-diversity was generally lower in the CAI than in the other strata, although in both years, this difference was statistically significant only in comparison with the BC stratum (Fig. 4). This result was consistent across the analytical approaches (Table S12).

3.2.2 Species turnover along the vertical gradient

Although a substantial proportion of species occurred throughout the entire vertical gradient (29% in 2024 and 40% in 2025; Fig. 5), the species shared among canopy and below-canopy strata were consistently more numerous than those shared with the CAI. The CAI shared the highest number of

species with UC in 2024 (77% of shared species) but, in 2025, shared species were highest with BC (73%).

In addition, the CAI harboured a large number of exclusive species, ranking second for species richness among the strata in 2024 (after the BC stratum) and first in 2025. Twenty-nine percent of these exclusive species were tourist species in 2024 and 19% in 2025.

3.2.3 Taxonomic and functional structure

Despite its distinct taxonomic composition, the CAI did not differ markedly from the other strata in terms of α-diversity. Indeed, local species richness was comparable in the CAI and the other strata in both years (Fig. 6). As a result, regional richness (γ-diversity) in the CAI was comparable to that of the canopy strata but lower than that of the BC stratum, although the difference was statistically significant in 2024 only (Fig. S15).

In both years, the abundance of rare beetles was higher in the CAI than below the canopy. The abundance and richness of rare species were both lower in the CAI than in the upper canopy in 2024. In 2025, rare species abundance was higher in the CAI than in the middle canopy.

Functional richness did not significantly differ between the CAI and the other strata in either year. Some patterns in the functional guild structure revealed several consistent distinctive features of the CAI assemblages. The CAI consistently harboured a greater abundance and richness of tourist species than did the other strata. The CAI also had a greater abundance of mycetophagous species than the below-canopy stratum. Conversely, saproxylophagous beetle richness was consistently lower in the CAI than within the canopy. Finally, the CWM of sciaphily did not vary among the strata in either year.

Metrics in bold are vertically stratified, with the stratum model fitting significantly better than the null model. Asterisks indicate models including spatial autocorrelation as a random effect. Detailed models are provided in Table S13.

Small black dots indicate non-significant differences between strata. Large red circles indicate significant positive effects, and large orange squares significant negative effects. Specific abundance and richness values are provided in Table S14.

3.2.4 Responses of abundant species

In 2024, a higher number of abundant species showed a preference for the CAI than for the below-canopy stratum, whereas in 2025 the number of abundant species preferentially associated with the CAI was comparable to the number of abundant species associated with the other strata (Fig. 7a).

The number of abundant species found predominantly in AC in at least one scenario is specified. The total number of abundant species by scenario is provided in Table S21.

3.3. Seasonal variation in the distinctiveness of the canopy-aerosphere interface

Beetle assemblages changed markedly throughout the sampling season in all the strata, reflecting pronounced seasonal turnover in community composition (Table S16). Although the CAI exhibited the lowest average dissimilarity between consecutive sampling periods (0.745), it showed the strongest effect of sampling period on community composition (PERMANOVA R^2 of 35.7%).

At the species level, adult activity periods differed markedly among species, with peaks of activity varying in both timing and duration (Fig. S17). Consequently, species were generally detected over only a short portion of the sampling season. At the plot level, a species was detected during an average of 1.28 sampling periods (1.41 after excluding singletons and doubletons). Across all the plots, the average detection time increased to 2.17 sampling periods (2.66 without singletons and doubletons; Table S18).

This rapid turnover of species throughout the season creates significant temporal variability in the stratification of our taxonomic and functional metrics. Indeed, all the taxonomic community metrics with the exception of conifer-associated species richness and all the functional metrics except for xylophagous species richness exhibited significant interactions between the stratum effect and the sampling period (Fig. S19 and Tables S19). Furthermore, several metrics that showed no significant vertical stratification when the entire season was pooled, e.g. mycetophagous species richness, sciaphily and preferred diameter substrate CWM, turned out to be significantly stratified when analysed through a finer temporal scale where sampling periods were distinguished.

Despite these pronounced seasonal changes, the CAI remained consistently distinct from the other forest strata throughout the sampling season (Table S20). Nevertheless, the degree of distinctiveness varied over time. Compositional dissimilarity between the CAI and the other strata reached its maximum in June, which also corresponded to the period when the differences between the canopy and the below-canopy strata were the greatest (Fig. 8a).

The * indicates the significance of the differences in intra-stratum heterogeneity values between AC and each of the other strata for each sampling event. Significance codes: *** < 0.001, ** < 0.01, * < 0.05.

Interestingly, whereas within-stratum heterogeneity remained stable or increased over the season in the canopy and below-canopy strata, assemblages sampled in the CAI became increasingly homogeneous over time (Fig. 8b).

The temporal changes in the distinctiveness of the CAI were also reflected by seasonal shifts in the vertical distribution of abundant species. Five abundant species out of the 14 preferentially associated with the CAI in April were more strongly associated with the upper canopy in May (Fig. 7b). From May onwards, however, the vast majority of the species associated with the CAI remained there the following months (64% between May and June and 70% between June and July-August). This trend was also true

for the other strata (Table S22). Most of the exchanges of preferred stratum that involved the CIA were with the UC, whereas exchanges involving the LC or the BC stratum were comparatively uncommon.

3.4. Impact of forest dieback on the distinctiveness of the canopy-aerosphere interface

The average dissimilarity among the canopy and below-canopy strata (UC, LC and BC) decreased (-7.5%) under declining conditions, whereas the dissimilarity between the CAI and the other strata changed only marginally (-1.4%; Table 2). While the community composition in the CAI remained significantly distinctive in the six most declining plots, the difference with UC was not significant in the six healthiest ones (permuted p-value of 0.3; Table S23).

Among the 22 taxonomic and functional metrics tested, the interaction between stratum and dieback was never significant.

Most of the abundant species exhibiting a clearly preferred stratum retained the same preferred stratum in both healthy and declining plots (60 out of 93 species, 64.5%). Among the species associated with the CAI in the healthy plots, 67.6% remained there under declining conditions. When shifts did occur among the strata, they were more often directed downward than upward (16 species shifted, 10 downward *vs*. 6 upward; Fig. 7c).

3.5. Impact of proximity to the vertical forest edge on the distinctiveness of the canopy-aerosphere interface

Proximity to the vertical forest edge had little influence on the distinctiveness of CAI community composition. Again, the average dissimilarity among the mid-canopy and below-canopy strata was lower (-5.6%) in the trees situated at the forest edges than in those situated at the centre of the forest islands, whereas the dissimilarity between the CAI and the other strata changed only marginally (-1.8%; Tables 2 and S23).

Nevertheless, the vertical stratification of some taxonomic and functional metrics varied according to tree position. Overall, common, saproxylophagous, zoophagous and floricolous saproxylic beetle species richness as well as zoophagous and floricolous abundance were vertically stratified only in the trees situated at the forest edge. In contrast, the abundance of rare and saproxylophagous beetles, the abundance and richness of conifer-associated and mycetophagous species, and mean adult body size were consistently stratified regardless of tree position (Fig. 6, Fig. S24 and Tables S24). Finally, total saproxylic beetle abundance, common beetle abundance, functional richness, xylophagous abundance and richness, and sciaphily CWM were vertically unstratified regardless of proximity to the vertical forest edge.

Despite the few metric-specific differences, species-level responses indicated a high degree of stability (Fig. 7d). Most abundant species with a clearly preferred stratum retained the same stratum regardless

of tree position (84 out of 116 species, 72%). Among the species associated with the CAI in the forest interior, 75.5% remained in the CAI at the forest edge.

4. Discussion

4.1. The canopy-aerosphere interface is a distinct forest stratum

Our study provides the first evidence that the CAI supports flying saproxylic beetle assemblages with levels of abundance and species richness comparable to those found within and below the forest canopy. These findings closely parallel those of Cunningham-Minnick et al. (2023), who showed that bee communities also occupy this aerial compartment, suggesting that the CAI could represent a biological habitat for multiple insect groups.

Beyond the mere presence of saproxylic beetles, our results demonstrate that CAI assemblages are consistently distinct from those sampled within and below the canopy, thus extending the previous descriptions of vertical stratification for saproxylic beetle communities restricted to below-canopy and canopy strata (Balvay et al., 2026; Bouget et al., 2011; Hagge et al., 2024; Ruchin et al., 2022). An additional distinctive compartment exists above the tree crown.

Beyond the mere presence of saproxylic beetles, our results demonstrate that CAI assemblages are consistently distinct from those sampled within and below the canopy, consistent with previous findings for bees (Cunningham-Minnick et al., 2023). This extends the previous descriptions of vertical stratification for saproxylic beetle communities restricted to below-canopy and canopy strata (Balvay et al., 2026; Bouget et al., 2011; Hagge et al., 2024; Ruchin et al., 2022) by showing that an additional distinctive compartment exists above the tree crowns for the corresponding taxa. Importantly, the differentiation of the CAI was not associated with reduced or increased local species richness but was primarily driven by species replacement. The low contribution of nestedness to Sørensen dissimilarity, together with the high number of exclusive species recorded in the CAI, indicate that the CAI assemblages cannot be regarded as a simple subset of canopy communities. Instead, the CAI is a distinct component of the forest vertical gradient with its own taxonomic identity.

This interpretation is further supported by the consistency of the patterns observed. Indeed, the taxonomic distinctiveness of the CAI was found in both sampling campaigns despite differences in the study sites and sampling design. Moreover, the pattern remained detectable throughout the season, under varying levels of forest dieback and regardless of tree position within the stand. Although our study was limited to mature, even-aged oak stands in the Loire Valley, the consistency of the patterns suggests that the differentiation of the CAI reflects intrinsic ecological characteristics rather than sampling artefacts or transient environmental conditions. Whether similar patterns occur in other forest types, climates or management systems remains to be investigated.

Recognising the CAI as a distinct forest stratum extends the classical view of forest vertical stratification that considers the upper canopy to be the limit of the forest habitat. Our results instead support the idea that forest biodiversity extends beyond the highest branches of the tree crowns into the interface with the aerosphere, where unique environmental conditions support distinct flying saproxylic beetle assemblages.

4.2. Why do saproxylic beetles use the canopy-aerosphere interface?

The presence in the CAI of flying saproxylic beetles raises a fundamental ecological question: what are these insects doing in a compartment that appears almost devoid of the dead wood resources on which they depend?

A first hypothesis is that the CAI acts as a dispersal corridor. Above the canopy, reduced physical obstructions and stronger airflows may facilitate movement over larger spatial scales (Finnigan, 2000; Wainwright et al., 2017). This has been demonstrated for *Dendroctonus ponderosae* (de la Giroday et al., 2011). Because the CAI provides an almost unobstructed aerial environment compared with the densely structured canopy, we expected larger-bodied species to be overrepresented - if reduced physical obstruction is a major driver of CAI use. However, this prediction was not supported: the beetles captured in the CAI were smaller on average than those captured in the upper and middle canopy, and comparisons with the below-canopy stratum varied between years. In fact, the CAI may actually favour small-bodied passive dispersers.

Several of our results support viewing the CAI as a transit zone. First, we consistently observed a higher diversity and abundance of conifer-only-associated species in the CAI compared with the strata situated within and below the canopy in our sampled oak trees in oak-dominated stands. The presence of these tourist species suggests that at least some individuals may represent transient beetles moving through the landscape rather than species specifically associated with the compartment. Future studies combining vertical sampling with species-level information on habitat affinity and landscape associations would help test this hypothesis. Indeed, saproxylic beetles are not restricted to continuous forests, and many species exploit scattered, patchy wooded habitats, such as isolated trees, hedgerows and orchards in agricultural landscapes (Dubois et al., 2009; Parmain and Bouget, 2018). Determining whether CAI assemblages contain a higher proportion of species associated with such fragmented or open wooded habitats would provide further evidence that the CAI is an aerial pathway facilitating movement among spatially-dispersed resources that connects multiple habitat patches at the landscape scale, a function recently highlighted for bee communities (Cunningham-Minnick et al., 2023). In our study, however, sciaphily CWM did not vary among strata.

Secondly, the lower spatial variability of CAI assemblages compared with the communities sampled within and below the canopy is another argument indicating the potential role of the CAI as a dispersal corridor. This pattern would be expected if the CAI channels individuals in transit rather than reflecting local, resource or microhabitat-driven assemblages. Unlike the lower strata, where community

composition is strongly shaped by tree species identity, crown architecture and local resource availability, a corridor used by dispersing individuals originating from multiple source habitats should favour homogeneous assemblages at different sampling points, thus diluting the site-specific signatures that dominate lower strata. The CAI environment itself is also conducive to homogenisation, being a more open aerial space characterised by strong airflows and greater atmospheric connectivity than in the more sheltered strata below (Finnigan, 2000). Interestingly, this relative homogenisation increased over the season, which would be consistent with a progressive accumulation of transient individuals arriving from an increasing diversity of source habitats as the season proceeds. While this interpretation remains speculative, it aligns with the broader idea that aerial compartments integrate insect movements over larger spatial scales than do strata directly tied to local resources.

Finally, the persistence of CAI distinctiveness under contrasting environmental contexts supports a dispersal-related interpretation. Several studies have shown that position in the forest influences vertical stratification in saproxylic beetles (Š. Vodka and L. Cizek, 2013; Sweeney et al., 2020; Wermelinger et al., 2007). Consistent with these findings, dissimilarity among the lower strata decreased under forest dieback and at forest edges, whereas CAI dissimilarity remained relatively stable. This suggests that the beetles occurring in the CAI may be less directly affected by local changes in tree condition or stand structure because some are simply moving through the landscape. This could mean that some beetles found in the CAI are on the go and are likely unrelated to the tree above which they were captured. Conversely, the lower forest strata host more resource-dependent species and therefore showed signs of biotic homogenisation under forest dieback (Balvay et al., 2026).

However, considering the CAI solely as a dispersal pathway would likely be an oversimplification. The presence of abundant and taxonomically distinct assemblages, including a consistent vertical preference for some dominant species, suggests that some species may actively exploit this compartment.

The CAI may provide favourable conditions for adult activity, particularly through increased solar radiation and warmer temperatures compared with lower forest strata (De Frenne et al., 2019; Tal et al., 2008; Vinod et al., 2023). Similar use of sun-exposed upper forest layers has been documented for several insect groups such as bees (Cunningham-Minnick et al., 2023) or butterflies (Mena et al., 2020). Although this mechanism remains poorly investigated in saproxylic beetles, warmer microclimates favour them (Bouget et al., 2013; Müller et al., 2015) and flying saproxylic beetle adults could potentially extend their daily activity periods or enhance their flight performance thanks to conditions in the CAI.

The CAI may also play a role in mate-finding and reproduction (Ulyshen, 2011). In several other insect groups, flight behaviour specifically dedicated to reproduction has been well documented: hilltopping in Lepidoptera (Alcock, 1987; Pe’er et al., 2013), swarming in Diptera (Downes, 1969) or drone congregation areas for bees (Otis, 2026). In saproxylic beetles, however, mate encounters appear to be more commonly associated with the resource exploited by both sexes and used for oviposition (Hanks, 1999) rather than with dedicated mating rendezvous sites.

The CAI may also provide temporary refuge from predators, parasitoids and competitors (Ferreira et al., 2014). Predation pressure on arthropods has been shown to decrease with increasing height in temperate forests (Aikens et al., 2013; Horchler and Morawetz, 2008). This could suggest that the CAI environment could be comparatively safer for flying insects. This hypothesis must be verified, however, as other conflicting studies have reported greater activity of insectivorous predators such as birds and bats within and above the canopy (Adams et al., 2009; Müller et al., 2013), where prey detectability may in fact be higher.

In any case, our results suggest that the CAI should not be seen as mere empty space above the canopy, but rather as a multifunctional aerial compartment used by saproxylic beetles for different purposes. Disentangling the relative importance of dispersal, microclimate use, reproduction, host searching behaviour, resource exploitation and predator or parasitoid avoidance will require complementary approaches, combining behavioural observations, flight tracking, chemical ecology and the sampling of potential aerial resources.

4.3. Seasonal turnover without loss of habitat distinctiveness

We expected strong seasonal changes in the ecological role of the CAI because vertical and temporal distributions of arthropods are closely linked to changes in resource availability and abiotic conditions (Wardhaugh, 2014). In our study, three processes may have operated simultaneously: the progressive development of the canopy, which modifies vertical gradients in light, temperature, humidity and wind across the strata (De Frenne et al., 2019; Lowman and Rinker, 2004); varying species-specific adult phenology; and the movements of the insects along the vertical gradient. The species we sampled are indeed characterised by highly diverse emergence periods and often brief adult activity, meaning that the composition of the flying assemblages changed continuously throughout the sampling period (Table S16 and Fig. S17).

The observed seasonal shifts in the preferred vertical position of abundant species confirm the influence of forest structure on the vertical stratification of the communities. Indeed, the marked redistribution of species between strata observed between April and May coincided with leaf emergence, which occurred in mid-April 2024. Leaf flushing profoundly modifies the physical structure of the canopy and strengthens vertical gradients in abiotic conditions. These contrasting abiotic conditions may explain why species exhibited greater changes in preferred stratum before canopy closure, whereas their vertical distribution became considerably more stable afterwards.

Our results nevertheless indicate that these temporal dynamics do not weaken the ecological identity of the CAI. Instead, though seasonal turnover did occur, a consistently distinct assemblage was maintained. This suggests that the CAI is not defined by the permanent presence of a particular set of species, but rather by ecological processes that repeatedly filter or attract different species sharing similar patterns of habitat use.

5. Conclusion

Our study demonstrates that the CAI is a full, though previously overlooked, component of temperate forest biodiversity. By providing efficient access to the entire vertical gradient of mature oak canopies over two consecutive activity seasons, the Canopix® enabled the first comprehensive characterisation of flying saproxylic beetle assemblages occupying this aerial compartment. The assemblages there proved to be taxonomically and functionally distinct from those sampled within and below the canopy, and remained consistently differentiated despite pronounced seasonal turnover, forest dieback and different distances from the vertical forest edge. Our findings highlight that, like microorganisms (Maki et al., 2023), bats (Müller et al., 2013) and bees (Cunningham-Minnick et al., 2023), saproxylic beetles actively use the CAI, supporting its recognition as an independent ecological compartment that may serve diverse functions, including movement, foraging, refuge, and mating. Consequently, biodiversity surveys of saproxylic beetle communities that are restricted to the forest interior inevitably underestimate their diversity and spatial organisation. Incorporating the aerial compartment into monitoring programmes should therefore improve assessments of forest insect diversity and strengthen our ability to detect ecological responses to environmental change, thereby contributing to more comprehensive conservation strategies for forest arthropods. Finally, our study focused on the CAI situated within the first few metres above the forest canopy. Extending sampling into higher atmospheric layers will be essential to determine how insect communities are structured above the canopy and to understand the role of the aerosphere in landscape connectivity, biodiversity monitoring and the conservation of forest arthropods.

Declarations

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Author Contribution

All authors contributed to the study conception and design. Material preparation and data collection were performed by R. Balvay, C. Couteau, G. Parmain and H. Martin. Analyses were performed by R. Balvay, C. Bouget and M. Laparie. The first draft of the manuscript was written by R. Balvay and all authors commented on the various versions of the manuscript. All authors read and approved the final manuscript.

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Data and code availability

Data and source code at the time of submission are available in the public archive repository Recherche Data Gouv at https://doi.org/10.57745/UQAS4C (to be made public after decision; private reviewer URL available at https://entrepot.recherche.data.gouv.fr/privateurl.xhtml?token=820e7482-20e1-40d7-90ec-b17e3715454f).

Supplementary information

The online version contains supplementary materials available at https://doi. org/10.XXXX/XXXXXX.

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Figures

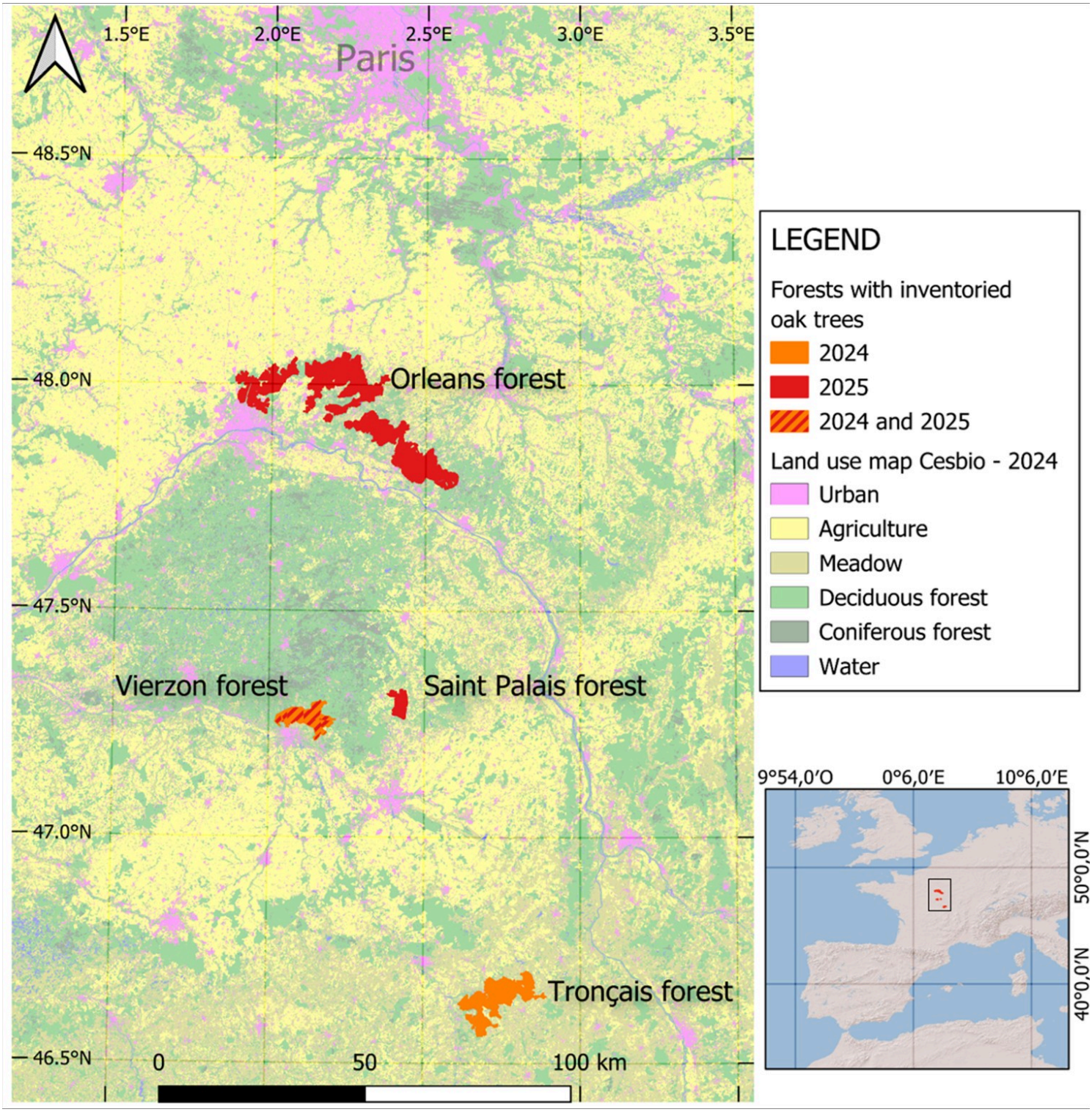


Figure 1

Map of the four forests in the Centre-Val de Loire region of France where our study sites were located : the Orléans, Vierzon, Saint Palais and Tronçais forests. Source of the land use map, Cesbio (Inglada et al., 2017)

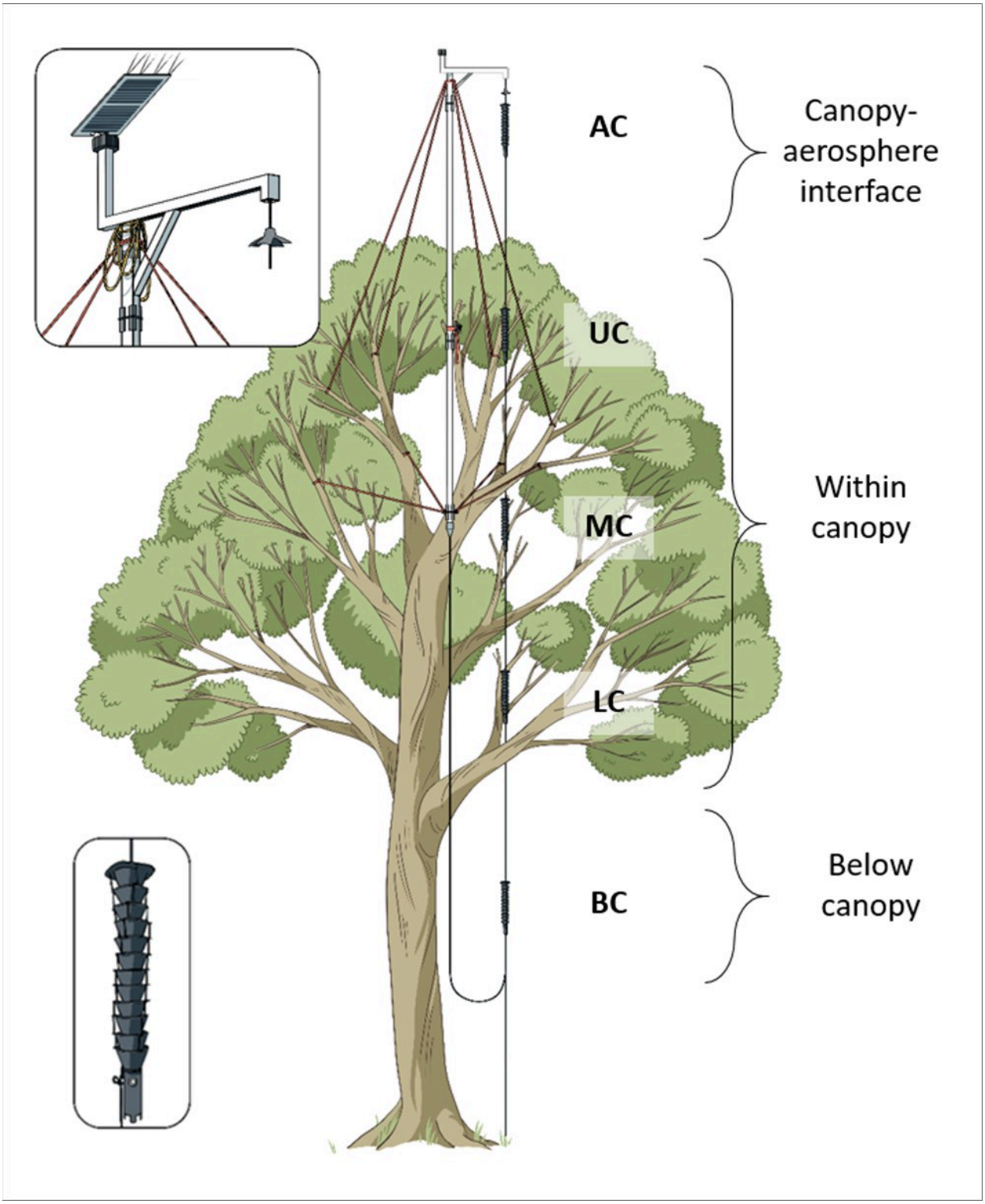


Figure 2

Illustration of a Canopix® installed in an oak, with the five sampled strata. Illustration by Aurélie Loyer

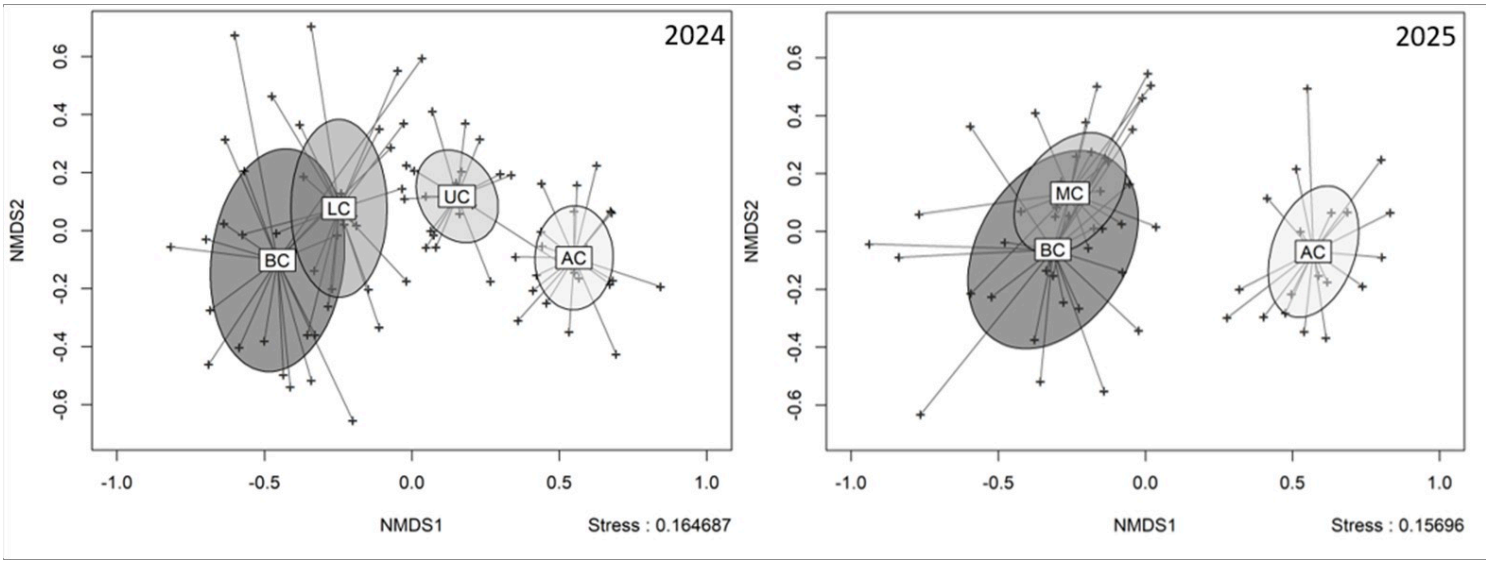


Figure 3

NMDS ordination based on Bray-Curtis dissimilarities, illustrating differences in species composition among the communities sampled at different vertical strata. Points represent the sampling sites and are connected to their stratum centroid by lines. Ellipses indicate one standard deviation around each group centroid.

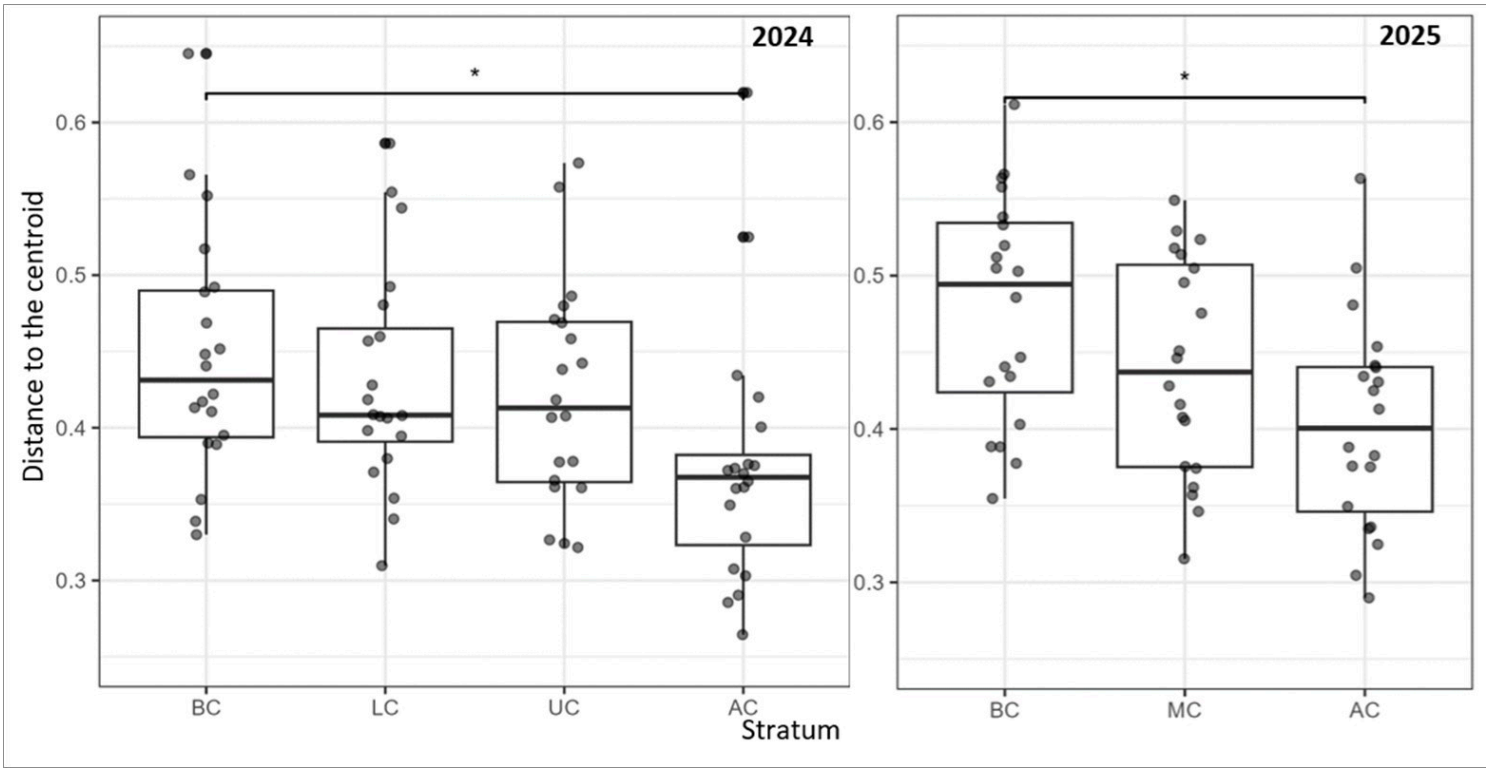


Figure 4

Differences in intra-stratum heterogeneity in 2024 and 2025

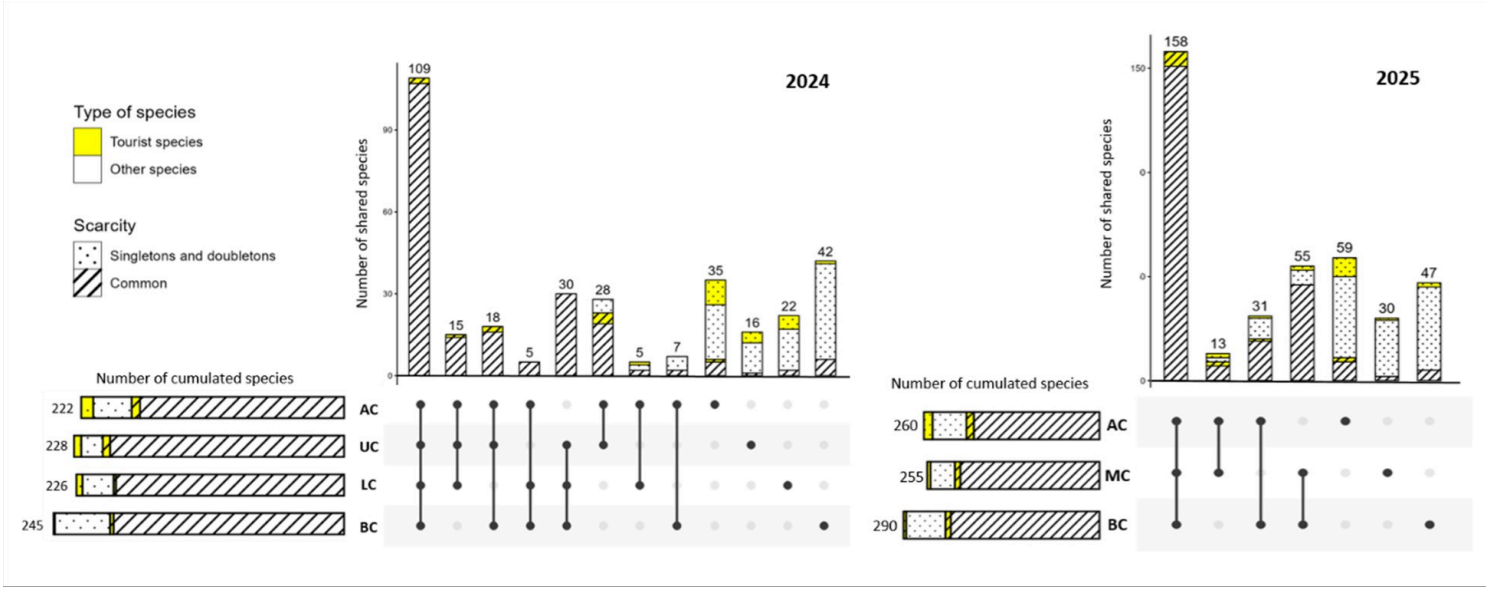


Figure 5

Number of shared species between strata

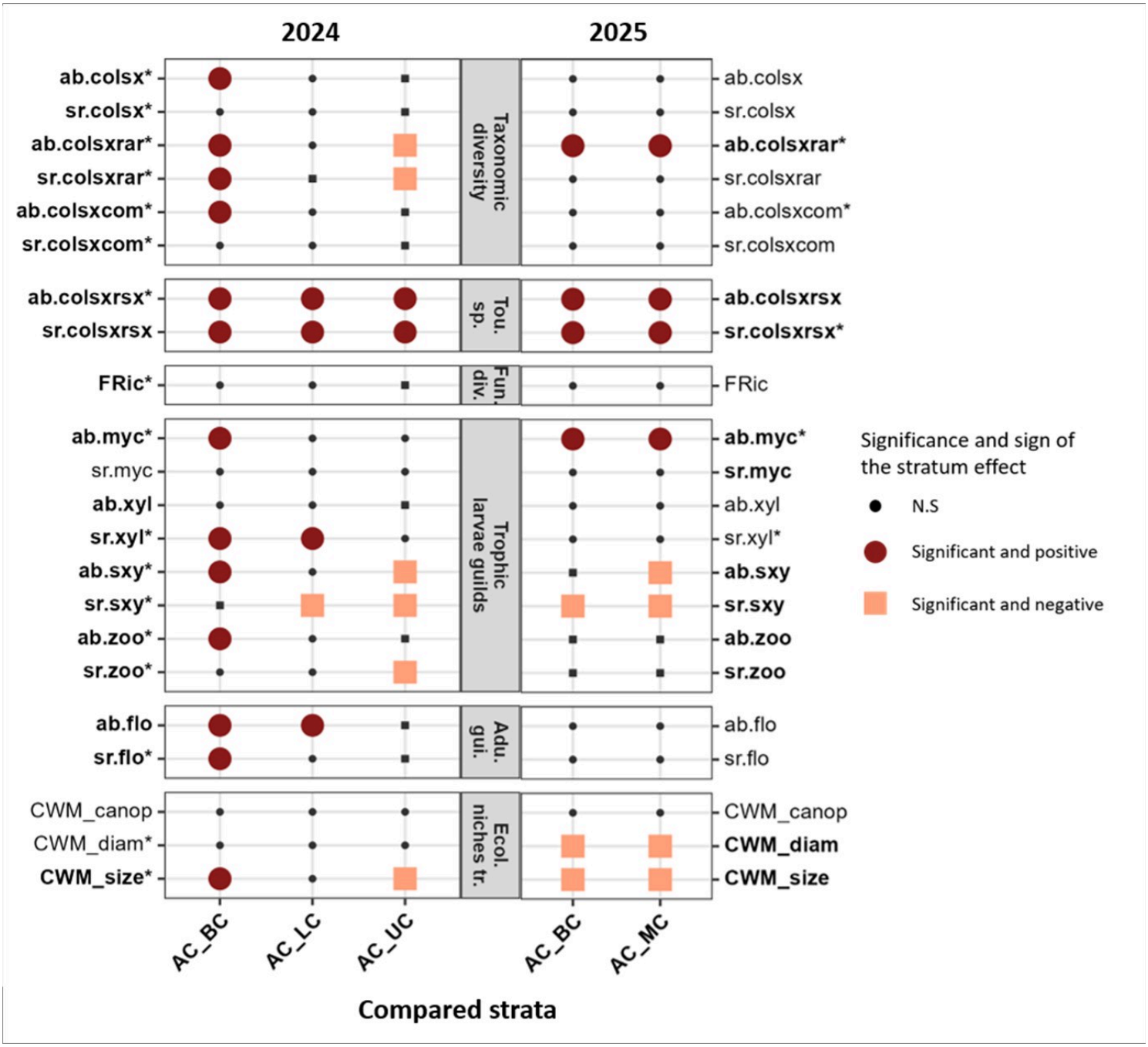


Figure 6

Summary of the GLMM results for the vertical stratification of taxonomical and functional entomological metrics (abbreviations in Table 1)

Metrics in bold are vertically stratified, with the stratum model fitting significantly better than the null model. Asterisks indicate models including spatial autocorrelation as a random effect. Detailed models are provided in Table S13.

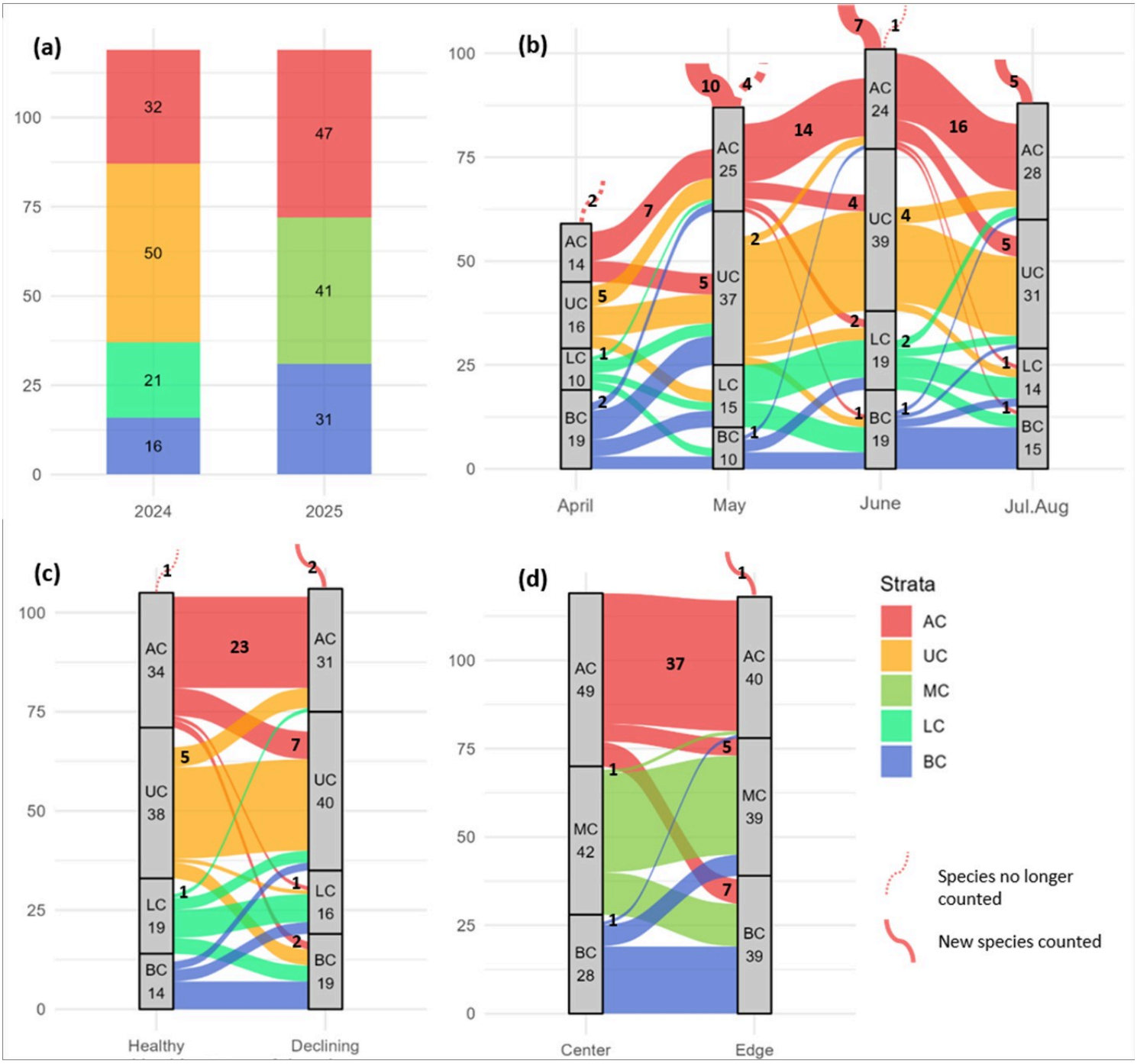


Figure 7

Changes in the number of abundant species predominantly sampled in each stratum, in terms of annual catch (a), and according to the sampling event (b), the health status of the plot (c) and the position of the tree within the stand (d)

The number of abundant species found predominantly in AC in at least one scenario is specified. The total number of abundant species by scenario is provided in Table S21.

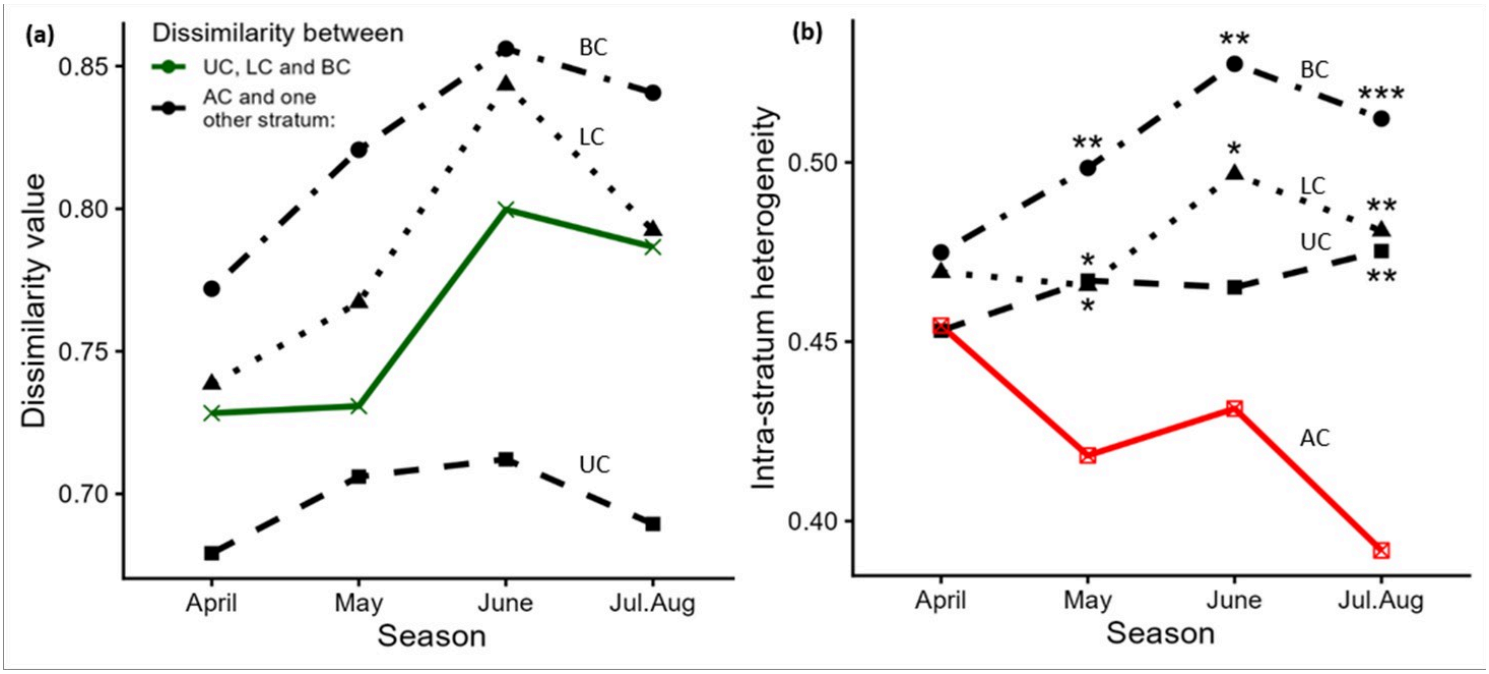


Figure 8

Changes in the average dissimilarity of AC compared with the other three strata (a) and within-stratum heterogeneities (b) during the trapping season

The * indicates the significance of the differences in intra-stratum heterogeneity values between AC and each of the other strata for each sampling event. Significance codes: *** < 0.001, ** < 0.01, * < 0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.pdf