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Avian nest-boxes enhance ant assemblage diversity across altitudinal and habitat gradients in Mediterranean woodlands

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ABSTRACT

1. Tree cavities create unique microclimatic conditions and, when occupied by animals, can accumulate organic materials that attract ants. However, their role in structuring ant assemblages across ecological contexts and their potential function as buffers against the effects of global change, remains poorly understood.

2. Here, we used nest-boxes as surrogates for natural cavities to test whether nest-box presence and bird use influence ant assemblages in Mediterranean woodlands. Using a paired design, we compared ant assemblage structure between trees with and without nest-boxes along a ~400 m gradient of altitude and habitat transformation measured as a decrease in natural vegetation cover. We also assessed whether birds affect nest-box microclimate and its altitudinal buffering.

3. Nest-boxes partially buffered external environmental variation relative to tree trunks, with those containing bird nestlings being warmer and drier. Compared to trunks, nest-boxes maintained more stable temperatures throughout the day and were generally more humid, particularly during early hours, although these differences diminished at higher altitudes and during warmer periods. Trees with nest-boxes supported ant assemblages that were taxonomically and functionally richer, and arboreal ant species occurred more frequently in trees with nest-boxes than in control trees. Phylogenetic diversity showed metric-dependent responses: neither Faith's PD nor mean pairwise distance (MPD) varied with nest-box presence; however, MPD increased with altitude in more transformed landscapes of lower vegetation cover and decreased in less transformed ones. However, we found no significant interaction between altitude, habitat transformation, and nest-box presence for any assemblage feature, indicating that nest-boxes did not function as ants' microrefugia. Neither nest-box occupancy by birds nor nest material type influenced ant assemblages.

4. Our findings suggest that nest-boxes primarily act as additional nesting sites that increase ant diversity in Mediterranean woodlands, rather than microrefugia shaping assemblages through microclimatic buffering. Nest-box supplementation may therefore influence tree-associated arthropod communities by modifying habitat availability.

***Keywords*:** ant diversity, altitude, environmental gradients, habitat transformation, Mediterranean ecosystems, microrefugia, nest-boxes, tree microhabitats.

INTRODUCTION

Predicting species responses to anthropogenic disturbance remains a major challenge. These disturbances often alter habitat conditions in ways that favor some traits over others, acting as environmental filters and thereby shaping communites. To persist, species need either functional traits or plasticity to overcome these novel conditions (McGill et al., 2006; Sunday et al., 2014). In this context, environmental filtering may be mitigated by microrefugia—(i.e. portions of habitat offering suitable conditions for diverse species (Gentili et al., 2015; Keppel et al., 2012)). For example, structural heterogeneity can buffer climatic extremes, particularly for ectotherms (González-Tokman & Dáttilo, 2024). This may be specially relevant in Mediterranean landscapes, where high biodiversity, steep environmental gradients, and chronic water limitations interact with strong sensitivity to climate change and human pressures (Doblas-Miranda et al., 2015; Klausmeyer & Shaw, 2009; Newbold et al., 2020).

Ants are among the most ubiquitous and functionally diverse organisms within ecosystems (Hölldobler & Wilson, 1990). They rapidly respond to disturbance, making them reliable indicators of ecosystem health (Folgarait, 1998; Ottonetti et al., 2006) and environmental change (Andersen, 1997; Arnan et al., 2014). Although mostly ground-dwelling, many ants also use trees as shelter (using beetle galleries or excavating their own (Villagran-Pinteño 1992, Novais et al. 2017) or as foraging areas (Klimes, 2017)). Trees act as ecological islands for ants (Adams et al., 2017; Gaytán et al., 2021; Yanoviak, 2015); and ant assemblages are shaped by interspecific competition (Dejean et al., 2007; Hölldobler & Lumsden, 1980; Sanders et al., 2007), tree traits (Cuissi et al., 2015; Klimes et al., 2012; Ribas et al., 2003) and refuge availability (McGlynn 2006; Peeters and Molet 2010; Jiménez-Soto and Philpott 2015). More broadly, habitat heterogeneity and microclimatic variation within trees are key drivers of arboreal ant diversity (Kirchner et al., 2025; Leahy et al., 2022).

Cavities in trees, including both natural hollows and artificial structures such as nest-boxes, can be a microhabitat structuring ant assemblages, as they likely provide hotspots for specialized interactions

(Avilés, 2024). Whether through trophic or thermal properties, cavities can offer specialized sites for diverse organisms, including bacteria (Berger et al., 2003; Goodenough et al., 2017), fungi (Korniłłowicz-Kowalska et al., 2018), and invertebrates (Machač & Tuf, 2021; Sunday et al., 2014; Turienzo, et al., 2010). Cavities often accumulate bird nesting materials and organic remains (e.g. droppings, ectoparasites (Brown et al., 2015; Lambrechts & Schatz, 2014; Salido et al., 2021), and even dead chicks or adults (Lambrechts et al., 2008; Smith et al., 2017)), which ants may exploit.

As ectotherms, ants may also benefit from the microclimatic conditions provided by bird nests in these cavities. In cool environment, breeding birds can create warm nest conditions that may favour ant brood development (Maziarz et al., 2020). In Mediterranean systems, however, where organisms are exposed to intense heat, ants may instead benefit from bird nests if these provide a buffered microclimate relative to the surrounding environment. Because environmental harshness can vary markedly along altitudinal gradients, the value of cavities as shelter may also depend on elevation. Yet, whether nest-boxes play such a role for ants remains unknown. Further, it is unclear whether ants use them opportunistically or preferentially. Some invertebrates show cavity specializations, while others use cavities depending on the resources they contain (Krištofík et al., 2003; Landry et al., 2013; Priest et al., 2021; Tajovský et al., 2001). Cavity use by birds could also scale up to community-level effects, as observed in the higher beetle richness in Barn Owl (*Tyto alba*) nests (Cosandey et al., 2021).

In Iberian holm oak (*Quercus ilex*) woodlands, the density of cavities suitable for cavity-dwelling species has been shown to be very low as a consequence of centuries-old management practices based on pruning and the removal of dead trees (Avilés, 2019; De la Hera & Avilés, 2026). In our study area, these management practices generate a gradient of habitat transformation ranging from relatively unmanaged stands with well-developed shrub layers to highly modified sites where shrubs have been largely removed and trees partially cleared for intensive crops (e.g. almond plantations). In this system, nest-boxes have been widely used as a tool to conserve and study communities of cavity-dwelling vertebrates (Lambrechts et al., 2008; Parejo et al., 2012; Parejo & Avilés, 2011). Although nest-boxes do not reflect the microclimatic variability of natural cavities, which are highly heterogeneous in size and shape (Amat-Valero et al., 2014; Maziarz et al., 2017), they have proven to buffer temperature

extremes (Goldingay & Thomas, 2021) and provide a standardized framework to study the effects on biological communities. Here, we use a long-term nest-box network on holm oaks in south-eastern Iberia to investigate how nest-boxes, and their use by birds, influence ant assemblages across a 400-m gradient of altitude and habitat transformation. Specifically, we used the percentage of shrub cover surrounding each sampling point as a proxy for land-use intensity, where higher shrub cover indicates less transformed conditions. Over two avian breeding seasons, we combined microclimate and bird monitoring, with surveys of ant assemblages on trees with and without nest-boxes to address two main goals: i) to determine whether nest-boxes modify microclimatic conditions along environmental gradients, thereby providing the mechanistic basis for a potential microrefugium effect; and ii) to test whether these microclimatic differences are associated with variation in ant assemblage parameters according to nest-box presence, occupancy, and nest type across environmental gradients (i.e. altitude and land-use intensity).

Ant diversity often declines with increasing altitude and under intense habitat transformation (de Queiroz et al., 2020; Guilherme et al., 2019; Machac et al., 2011; Tylianakis et al., 2008). In this context, the hypothesis that nest-boxes function as microrefugia predicts an interaction between these environmental gradients and nest-box presence on ant assemblage traits, with nest-boxes providing favourable conditions by buffering the abiotic filters imposed by altitude and land-use intensity. Specifically, we predict that this buffering effect will promote higher species richness under harsher environmental conditions in trees with nest-boxes compared to trees without nest-boxes. Given that the studied ant assemblages comprise relatively few functional groups and genera (Salido et al., 2024, Table S3), we further expect this effect to be more pronounced for taxonomic richness than for functional or phylogenetic diversity. In addition, bird occupancy can alter cavity microenviromental conditions and modify microclimate, with effects that may vary among bird species depending on factors such as prey remains in nests, incubation, nestling periods or nest type. Accordingly, we expected ant assemblages to differ with nest-boxes presence and their occupancy.

MATERIALS AND METHODS

Study area

The study area is located in the surroundings of Sierra de Baza (Granada, Spain, 37°18′N, 3°11′W), in an extensive agricultural landscape of ~22 km2 dominated by scattered holm oaks. Between 2010 and 2012, ~550 cork-made nest-boxes have been randomly installed in holm oaks, supporting a breeding community of little owls (*Athene noctua),* scops owls (*Otus scops)*, European rollers (*Coracias garrulus)*, common hoopoes (*Upupa epops)*, great tits (*Parus major)*, jackdaws (*Corvus monedula)* and stock doves (*Columba oenas*) (Avilés et al., 2019; Parejo et al., 2012; Parejo & Avilés, 2011). Nest-boxes (24 × 24 × 40 cm; 6-cm entrance) were cleaned before bird breeding. The climate is semiarid Mediterranean, with sharp seasonal and daily temperature fluctuations, winter rainfall, and dry summers with occasional storms (Díaz-Hernández et al., 2003).

Altitudinal gradient and microclimatic variation

Nest-boxes are distributed along a 400-m altitude gradient (996-1415 m). To assess the extent of microclimatic variation across this gradient, we used 100-m resolution raster layers of maximum and minimum temperature and relative humidity (2013-2023) from the Andalusian Environmental Information Network (REDIAM). Mean June values, corresponding to the ant sampling period, were extracted for 490 random points (~number of sampled trees) and processed using QGIS (QGIS Development Team 2024).

Nest-boxes and microclimatic variation

In 2023, we used temperature and humidity data loggers (Hygrochron DS1923-F5, iButton ®, Dallas Semiconductor, USA) recording every 30 min to register microclimatic variation in nest-boxes. Loggers were installed in plastic holders fixed to the nest-box base, and paired with “control” devices on trunks. We characterized microclimate in 23 nest-boxes and 16 controls, as nearby trees with nest-boxes shared a single control. Loggers were randomly distributed with respect to altitude (range for nest-boxes with loggers: 1004–1388 m) in nest boxes occupied by birds, making this microclimatic assessment representative of the variation within our study area. Devices were installed in May in active nests and

remained until the end of the nesting period, which extended to mid-July for the latest-hatched scops owl nests. Measurement duration varied among devices due to differences in nest detection, occupying species, and nest failures. Using this data, we tested whether microclimate differed with nest stage between occupied (eggs/chicks) and predated (no active breeding resembling a control situation), and whether nest-boxes buffered altitudinal microclimatic variation.

Habitat transformation gradient

The study was conducted along an agricultural intensification gradient dominated by holm oaks. In this system, habitat transformation is primarily driven by the progressive removal of shrub vegetation and, to a lesser extent, tree cover, resulting in a transition from structurally complex, less-managed stands to simplified and intensively managed areas (e.g. almond plantations).

We analysed ant assemblages at two scales: locally**,** as the percentage of Mediterranean shrubland within a 10-m buffer around each tree (i.e. local vegetation cover); and at the landscape level, as the percentage within a 100-m buffer (i.e. landscape vegetation cover). Shrub cover was used as a proxy for habitat transformation, with higher values indicating less disturbed conditions, and lower values representing more intensively managed habitats. Measurements were obtained for all trees with nest-boxes and their paired neighbors.

Nest-box monitoring

During the two-year study, nest-boxes were checked biweekly from mid-April to late June to record bird occupancy and breeding species. Based on these surveys, nest box occupancy was defined as three-level categorical variable: "previously occupied" (presence of nest remains indicating recent breeding activity, either successful or failed), "currently occupied" (active nest with eggs or chicks), and "never occupied" (no evidence of breeding activity).

Occupied nest-boxes were further classified according to nest type, based on the structural characteristics of the nest and the amount of nest material present. We distinguished three categories: nests without additional nest material (e.g., *A. noctua*, *C. garrulus*, *O. scops*, *U. epops*), nests with sparse

material (*C. oenas*), and nests with well-formed cup structures (*C. monedula*, *P. major*). We assumed that nest structure, rather than bird species identity, was the main driver of variation in ant assemblages.

Ant sampling and assemblage metrics

We randomly selected 100 trees with nest-boxes and paired each with the nearest neighbor. The mean distance between a tree with a nest-box and its nearest neighbor was 21.97 m (range: 5.3–57.9 m), while the mean distance between trees with nest-boxes was 79.68 m (range: 25.3–860.12 m). Although ant assemblages can vary between trees within a pair even at close proximity (Adams et al., 2017), this design ensures that paired trees share similar altitude and habitat transformation levels. Therefore, any observed differences in ant assemblages can be attributed primarily to the presence of nest-boxes, which had been in place on the trees for at least 10 years before ant sampling. Pairs were selected in 2022, and resampled in 2023.

Ant assemblages were sampled using arboreal pitfall traps placed on each tree during the first half of June each year, coinciding with the middle of the birds' reproductive season. Traps consisted of a plastic cup (≈ 100 ml) nailed to the trunk next to the nest-box and at a similar height (2-3 m) on neighboring trees. We avoided sampling ants inside the nest-boxes to prevent disturbing the birds and potentially causing nest abandonment. Accordingly, we did not use in-box pitfall traps or conduct additional systematic inspections of nest interiors beyond the routine nest visits associated with bird monitoring. Traps contained a sugar solution (4.67g/L) with soap (Delabie et al., 2021), were collected after 48 h, and subsequently frozen. Previous studies have shown that this sampling duration is effective for characterizing both diurnal and nocturnal arboreal ant assemblages (Almeida et al., 2023; Salido et al., 2024). Ants were preserved in 96º ethanol for species identification (Bolton, 2022) at the Ecology lab of the University of Córdoba (Spain). Figure 3 shows trap detection rates per species and worker abundance in trees with and without nest-boxes.

Given the low number of species per sample (range 1-9), we calculated richness as the simple number of ant species per pitfall trap (*specnumber* function from the vegan package; Oksanen et al., 2022).

Functional groups followed Roig & Espadaler (2010), except *Camponotus cruentatus* and *Crematogaster scutellaris*, reclassified as coarse woody debris specialists (CWDS) due to their association with holm oaks (Jiménez-Carmona, 2021). In addition, species were classified as arboreal when they nest preferentially in deadwood or within the arboreal stratum (Lebas et al., 2017, Table S3).

Functional group richness per pitfall was then calculated as the number of functional groups represented among the workers recorded in each trap.

For phylogenetic measures, we used a phylogenetic tree based on the European ant species by Moreau & Bell (2013) assembled by Xavier Arnan and Xim Cerdá (Figure S1). For each pitfall, we calculated vi) Faith's PD as a measure of evolutionary history represented by the species and vii) Mean Phylogenetic Distance (MPD), defined as the mean pairwise phylogenetic distance among all species (*pd* and *mpd* functions in the picante package Kembel et al., 2020).

Statistical Analyses

All statistical analyses were performed in R environment (version 4.2.1; R Core Team, 2022). Model diagnostics included visual inspection of residuals *vs.* fitted values and overdispersion tests (*check_overdispersion* function in performance package; Lüdecke et al., 2021). Numeric predictors were standardized to 0 mean and variance 1 using the *scale* function. *P*-values were obtained via Type III ANOVA (*Anova* function in car package; Fox and Weisberg 2024) and visualizations were created with ggplot2 and sjPlot packages (Lüdecke et al., 2024; Wickham, 2016).

Altitude and Microclimatic Variation

To assess how altitude influenced climatic conditions, we used linear models (*lm* function) to test the relationships between altitude and microclimatic variables (i.e., maximum/minimum temperature, humidity).

We also examined microclimatic variation in relation to nest stage, included as a three-level fixed factor: nests containing eggs (incubation stage), nests with chicks (nestling stage), and predated nests (no active breeding resembling a control situation). We fitted two generalized linear models with Gaussian error

distribution (using the *glmmTMB* function in glmmTMB package; Brooks et al., 2024), one for temperature and one for humidity. Fixed effects included bird species, nest stage, laying date (Julian day), and the species × nest stage interaction. Two nests (one of *C. monedula* and one of *U. epops*) were excluded because they were the only nests available for those species, preventing reliable parameter estimation.

We further analysed variation in temperature and humidity using two generalized linear mixed models with Gaussian error distribution fitted with *glmmTMB*, with data logger location (nest-box *vs*. trunk) as a fixed effect, and Julian date, time (hour), and altitude as covariates. To test whether differences between nest-boxes and trunks varied along the altitudinal gradient and across seasonal and daily temporal variation, the models also included three-way interactions (location × date × altitude and location × time × altitude), as well as all two-way interactions involving location and altitude. Logger ID was included as a random effect nested within the ID of each pair (nest-box- neighboring tree). Because data loggers recorded temperature and humidity every 30 min throughout the breeding period, the analyses were based on repeated measurements from each logger.

Variation in Ant Assemblages

First, we evaluated differences in community species composition between trees with nest-boxes and trees without nest-boxes using a non-metric multidimensional scaling (NMDS) ordination implemented with the function *metaMDS* in the vegan package. The statistical interference was then tested using ANOSIM (function *anosim*, package: vegan). The analysis was based on species-level abundance data, using the number of worker ants collected in pitfall traps for each treatment (trees with nest-boxes vs. trees without nest-boxes) for both years.

We then analysed taxonomic, functional, and phylogenetic metrics of ant assemblages at three levels: i) presence of a nest-box in a tree (nest-box vs. neighboring trees without nest-box), ii) nest-box occupancy status, and iii) nest type. For each of these three analyses, species richness was modelled using a Poisson error distribution with a log-link function, whereas all functional and phylogenetic diversity metrics were analysed using Gaussian models. All models were fitted using the *glmmTMB* function.

Models included year as a fixed factor, as well as altitude, local vegetation cover, and landscape vegetation cover, including all possible two-way interactions among these variables. Group ID (which grouped each tree with a nest-box and its nearest neighbouring tree without a nest-box) was included as a random effect to account for the paired study design.

The effect of nest-boxes occupancy by birds on the ant assemblages was analysed was analysed only for trees with nest-boxes, as neighbouring trees without nest-boxes cannot be occupied. Consequently, the paired design was not applicable at this level, and occupancy effects were analysed using generalized linear models. Occupancy status, year, altitude, local vegetation cover, landscape vegetation cover, and their two-way interactions with occupancy status were included as fixed effects.

Finally, we analysed whether assemblage metrics varied with nest type using nest-boxes occupied by birds in both years (n=34). Models included nest type, year, altitude, local vegetation cover and landscape vegetation cover as fixed effects. No interactions were tested due to limited sample size.

RESULTS

Altitude, nest-boxes and Microclimatic Variation

Maximum temperature, minimum temperature, and relative humidity decreased with altitude (Figure S2). The maximum and minimum temperatures at lower altitudes were ~2.5°C and 2.0°C higher, respectively, and the relative humidity 6% higher than at higher altitudes (Figure S2).

Data logger analyses revealed that nest-box microclimate varied with the interaction between bird species and nest stage (z=-1.42, p=<0.001; Table S1). Across species, nest-boxes with chicks were warmer and drier compared to those with eggs, whereas predated nests showed sharp decreases in temperature (e.g. *O. scops* ~10 °C cooler) and higher humidity (e.g. *O.scops* ~23% more humid; Figure S3).

Analyses of interior-exterior data loggers showed a three-way interaction among location, altitude, and time of day on temperature (z = -5.17, p=<0.001; Table S2). Temperature decreased with altitude both

inside occupied nest-boxes and trunks (Figure 1a). However, daily temperature variation with time of day remained constant inside nest-boxes but declined on trunks (Figure 1a).

Figure 1 about here

A significant location x date interaction also emerged (z=-3.97, *p*=0.001; Table S2): in spring, interior and exterior temperatures were similar, whereas in summer, when temperatures peaked, nest-boxes were warmer (Figure S4).

Both humidity models showed significant three-way interactions (location x altitude x time of day: z=11.36, *p*=<0.001; location x altitude x date: z=17.33, *p*=<0.001; Table S2). Early in the day, occupied nest-boxes were more humid than trunks across all altitudes (Figure 1b). At midday and in the afternoon, trunk humidity increased with altitude, reducing differences with nest-boxes (Figure 1b). At lower altitudes, nest-box humidity remained stable over time, while trunk humidity declined (Figure 1c). Conversely, at higher altitudes, temporal humidity differences in trunks were amplified within nest-boxes (Figure 1c).

Ant assemblage in holm oak trees

Most pitfalls contained ants (349 of 400; 87.25%), yielding a total of 19.194 individuals and comprising 24 species (Table S3). Six of these species are arboricolous (Table S3). *Camponotus* was the most diverse genera of our assemblage, represented by eight species. In terms of species abundance, *C. cruentatus* was found to be the most abundant species of the entire assemblage, regardless of whether the tree had nest-box or not. In holm oaks with nest-boxes, the most abundant species was *Colobopsis imitans*, followed by *C. sylvaticus* and *Iberoformica subrufa,* whereas *Crematogaster auberti* and *C. piceus* were more abundant in the neighboring holm oaks without nest-boxes. *Cataglyphis iberica*, *Crematogaster sordidula*, *Tetramorium semilaeve*, *Temnothorax racovitzai* were found in a low abundance and exclusively in holm oaks without nest-boxes (Figure 3, Table S3). Species also varied widely in the altitude and landscape vegetation cover ranges they occupied (Figure S5). For instance, *C. cruentatus* were mostly found at higher altitudes (1200-1400 m, Figure S5a) and consistently in sites with greater landscape vegetation cover (Figure 5b), whereas *C. scutellaris* occurred across a broader

altitudinal range (1032–1400 m, Figure S5a) and in sites spanning both low and high vegetation cover (Figure S5b).

When species were grouped according to their ecological affinity (Table S3), arboreal species showed higher occurrence in trees with nest-boxes than in paired control trees, whereas no comparable pattern was observed for non-arboreal species (Mann-Whitney test, W=104.5, p=0.0007, Figure 3). However, community composition did not differ significantly between trees with nest-boxes and trees without nest-boxes, as indicated by the ANOSIM (R=0.006, p=0.094).

The detected species were assigned to five functional groups: cold-climate and shade-habitat specialists (CCS, 37.5%), generalists and opportunistic species (GO, 25%), hot-climate and open-habitat species (HCS/OH, 25%), coarse woody debris specialists (CWS, 8.3%) and cryptic (C, 4.1%) (Table S3).

Variation in ant assemblages in relation to nest-box presence, altitude and habitat transformation

Ant assemblage varied with nest-box presence, altitude, and local vegetation cover (Table S4). Richness was marginally higher in trees with nest-boxes (nest-box presence: β=0.12±0.06, z=1.95, p=0.05; Figure 2a), and declined with altitude (altitude effect: β=-0.19±0.08, z=-2.49, p=0.01; Figure S6). Richness also decreased from the first to the second year of the study (year: β=-0.44±0.07, z=-6.62, p<0.0001, Table S4).

Figure 2 about here

Functionally, richness was likewise higher in trees with nest-boxes (nest-box presence: β=0.06±0.03, z=2.14, p=0.03; Figure 2b) and in less transformed landscapes (landscape vegetation covert: β= 0.17±0.06, z=2.74, p=0.006; Figure S7 and Table S4). Functional richness declined between years (year: $\beta = -0.20 \pm 0.03$, $z = -7.07$, $p < 0.0001$; Table S4).

At the phylogenetic level, MPD depended on the interaction between altitude and landscape transformation (z=-2.72, p=0.007; Table S4): MPD increased with altitude in more transformed landscapes and decreasing in less transformed ones (Figure S8). Phylogenetic diversity (PD) varied only with year, showing lower values in 2023 (year: $\beta = -0.15 \pm 0.04$, $z = -3.47$, $p < 0.001$; Table S4).

Variation in ant assemblages in relation to nest-box use and nest type

The taxonomic, functional, and phylogenetic traits of ant assemblages did not differ between nest-boxes based on their use by birds (Table S5), and this lack of pattern is not affected by altitude or by habitat transformation (Table S5). Likewise, no differences were detected between nest types (Table S6).

DISCUSSION

Our study shows that nest-boxes act as important tree microhabitats that may potentially buffer microclimatic variation and promote richer, more functionally diverse ant assemblages, even under local low levels of vegetation cover. The overall ant use of nest-boxes was largely independent of birds, and community composition remained broadly unchanged, suggesting that ants exploit nest-boxes as microhabitats rather than responding specifically to bird occupancy. In what follows, we discuss nest-boxes as enhancers of habitat availability and the influence of environmental gradients.

Nest-boxes in trees as enhancers of habitat availability for ant assemblages

Scattered trees contribute to landscape heterogeneity, promoting the conservation of insect diversity in otherwise simplified landscapes (Dunn 2000, Reyes-López et al. 2003). In Mediterranean holm oak woodlands, areas with trees support greater ant richness than open grasslands (Gaytán et al., 2021). Here, we found that adding nest-boxes in trees increased ant species and functional richness. However, these effects were not accompanied by detectable shifts in overall species composition, suggesting that nest-box addition primarily enhanced habitat use by existing assemblages rather than driving species turnover. Tree-associated ant assemblages comprise both strictly arboreal and ground-nesting species (Table S3), which likely differ in their dependence on cavities. Consistent with this idea, arboreal species were generally more frequent on trees with nest-boxes than on paired control trees (Figure 3), suggesting that species more closely associated with tree cavities may benefit more from the additional nesting substrate. More generally, given the scarcity of natural cavities in dehesas (Avilés, 2019), artificial cavities may act as key tree microhabitats, enhancing structural complexity and providing additional microhabitats for ants.

One likely mechanism behind these effects on ant assemblages is the buffering capacity of nest-boxes to moderate environmental variability (Martin & Ghalambor, 1999; Sedgeley, 2001), which may be relevant for brood development (Maziarz et al., 2020). Our microclimate analyses revealed that, while both trunks and nest-boxes cooled with altitude, daily temperature variation remained smaller in nest-boxes than in trunks at lower altitudes. Similarly, nest-boxes exhibited more stable humidity levels than trunks during early morning and across dates at lower altitudes. Altogether, this points to the buffering effect being more pronounced at elevations below 1200 m, where most ant species occur. Further work is needed to clarify the conditions under which nest-boxes may provide stable microclimatic buffering for ants. Nevertheless, the highly variable patterns of temperature and humidity between nest-boxes and trunks suggest that any potential role of nest-boxes as microrefugia in our study site is likely to be temporally limited, occurring only at specific times or conditions rather than consistently over time.

Beyond microclimate, nest-boxes may also provide shelter and predictable food resources, including avian debris, carrion (Salido et al., 2024; Smith et al., 2017) or ectoparasites (Salido et al., 2021). Yet, the structure of ant assemblages did not differ according to bird use, nest types or habitat transformation. Likewise, multivariate analyses revealed no significant compositional differences associated with nest-box addition. Thus, ants seem to exploit nest-boxes regardless of origin or occupancy, likely responding to structural or microclimatic features. For arboreal ants such as *C. scutellaris*, which we observed relocating brood within nest-box walls, nest-boxes may serve as key refuges, particularly under thermally variable conditions that affect brood development (Hartley & Lester, 2003; Porter, 1988). The absence of an effect associated with bird occupancy may result from the pre-breeding cleaning of nest-boxes (see Methods), which removed materials that could have favored ants. In addition, we cannot discard that the absence of a nest-type effect was due to low power of the analysis (n=34), or the fact that the pitfall design outside nest-boxes is simply insensitive to within-cavity occupancy effects. In any case, our results suggest that ant assemblages were not significantly influenced by either the enrichment of nest-boxes resulting from bird activity or the microclimatic stabilization associated with their occupancy.

Nevertheless, the increase in taxonomic and functional diversity observed in trees with nest-boxes was not accompanied by changes in phylogenetic diversity. This pattern could be explained by the generally low phylogenetic diversity of our assemblages, driven by the limited number of species and the dominance of a few genera, such as *Camponotus*. In this context, cavities may increase habitat heterogeneity and allow species with different ecological niches to establish, but without recruiting lineages that are more distantly related. In other words, the observed effect seems to reflect a stronger response to microhabitat availability than to the deep evolutionary history of the species.

An additional source of variation was the strong decline in most ant responses between the two sampling years. Sampling was conducted on the same dates in both years to ensure temporal comparability; however, spring phenology differed markedly between years. In the second year (2023), spring was delayed, with colder and wetter conditions persisting into June. Such conditions may reduce ant activity and foraging, potentially affecting their detectability and occurrence at the time of sampling. Thus, the strong interannual variation observed in our data may reflect differences in seasonal environmental conditions rather than changes in the effects of nest-boxes or the environmental gradients examined. This result also highlights the importance of temporal variation in shaping ant assemblages in Mediterranean woodlands.

Effect of the altitudinal and habitat transformation gradients on ant assemblages

Variation in ant assemblages along environmental gradients is well documented (Arnan et al., 2014; Dunn et al., 2010; Sanders et al., 2007). In relation to altitude, two general patterns have been described: a monotonic decline and mid-altitude richness peaks (Dunn et al., 2010; Sanders et al., 2007). Our results align with the former, with richness decreasing with altitude even across the relatively narrow gradient of our area. Microclimate analysis confirmed declines in temperature and humidity with elevation in our study site (Figure S2), which may act as abiotic filters influencing ant physiology, activity, and nesting opportunities (Körner, 2007; Sundqvist et al., 2013), showing that even fine-scale elevational differences might shape assemblage structure.

We found that functional group richness was higher in trees surrounded by greater landscape vegetation cover, consistent with previous studies showing that ant functional diversity is positively associated with habitat complexity (Arnan et al., 2018; Hevia et al., 2019). Our results highlight the importance of shrub cover at the landscape scale as a key factor promoting functionally richer ant assemblages in holm oak woodlands. Most ant species were recorded mainly in intermediate to highly vegetated landscapes, whereas they were less frequent in the most transformed areas (Figure S5b). In particular, *T. racovitzai* occurred only in sites with higher landscape vegetation cover, whereas other species, such as *C. iberica* or *C. sordidula*, occurred only in less vegetated landscapes (Figure S5b). Although previous studies demonstrated that altitude and habitat transformation can interact to influence insect diversity (Liao et al., 2024), we did not detect such an interaction for ants in our system. This absence of an effect may reflect the limited altitudinal range of our study area or differences in the taxa considered compared to other studies.

Regarding phylogenetic metrics, we found an interaction between altitude and landscape vegetation cover. MPD increased with altitude in areas with lower vegetation cover, but declined with altitude in more vegetated landscapes. This pattern may indicate that the balance between environmental filtering and habitat buffering changes across the landscape. In more open, less vegetated areas, increasing altitude may impose stronger abiotic constraints that favor the persistence of a smaller set of phylogenetically distinct taxa, thereby increasing MPD. By contrast, in landscapes with greater vegetation cover, the buffering effect of the surrounding habitat may moderate climatic stress and promote communities composed of more closely related species, leading to lower MPD at higher altitudes. This interpretation is supported by the species distributions in our community (Figure S5), which vary non-randomly across altitude and landscape vegetation cover, even within the most species-rich genus, *Camponotus*. Although phylogenetic diversity is expected to decrease with altitude (Smith 2015), Mediterranean ant assemblages have not always shown a clear phylogenetic pattern in relation to environmental gradients (Silvestre et al., 2021). In contrast, similar to our results, ground-dwelling ant assemblages in the Brazilian caatinga, showed higher diversity at intermediate altitudes under reduced disturbance and milder abiotic stress (Silva et al. 2024).

While vegetation cover around trees is an important factor influencing ant assemblages (Lach et al., 2010), our findings suggest a stronger effect of landscape vegetation cover in holm oak woodlands of the southeast of Spain.

Conclusions

Our findings show that nest-boxes in holm oaks function as additional microhabitats, enhancing tree structural heterogeneity and providing novel ecological niches for ants. These effects were mainly reflected in increased richness and functional diversity rather than in major changes in species composition. Ant use of nest-boxes seems independent of birds, suggesting opportunistic coexistence. By offering additional cavities in areas where natural hollows are limited, nest-boxes contribute to habitat enrichment, increasing both structural complexity and resource availability. Our decade-long experiment, in which artificial cavities have been maintained for over ten years, demonstrates that nest-box provisioning can induce long-lasting effects on ant assemblages, highlighting artificial cavities as both conservation tools and experimental systems for studying ecological plasticity in dynamic landscapes. Also, our findings have practical implications for the management of Mediterranean woodlands, as nest-box provisioning may help maintain structural complexity and promote local insect diversity in landscapes where natural tree cavities are scarce.

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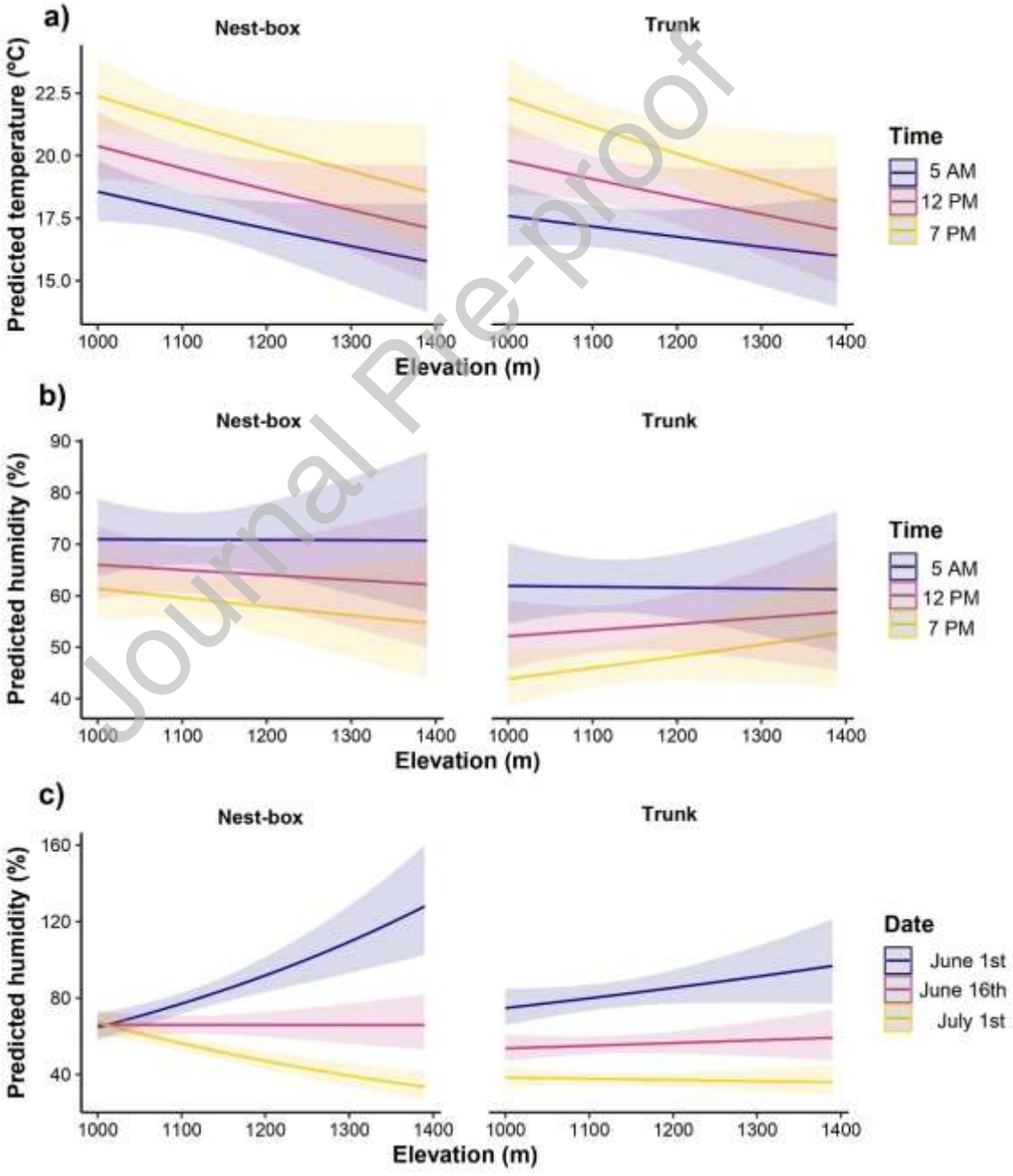
Figure 1. **Microclimatic variation in the interior of nest-boxes and tree trunks in relation to date, time and altitude.** a) Predicted temperature in nest-boxes and trunks in relation to time and altitude; b) Predicted relative humidity (%) in nest-boxes and trunks in relation to time and altitude; c) Predicted relative humidity (%) in nest-boxes and trunks in relation to altitude and date. Predictions are derived from GLMs (Table S2). We used different scales for humidity to facilitate the visualization of the effects.



Time and date groups with different colors correspond with the mean and standard deviation. The shaded areas represent 95% confidence intervals.

Figure 2. Variation in ant community in relation to nest-box presence. Values are predicted for richness, and functional richness from GLMMs (Table S3). a) Ant richness in trees with and without nest-boxes; b) Functional richness in trees with and without next-boxes. Vertical bars represent standard errors. Functional richness was log-transformed to generate model predictions.

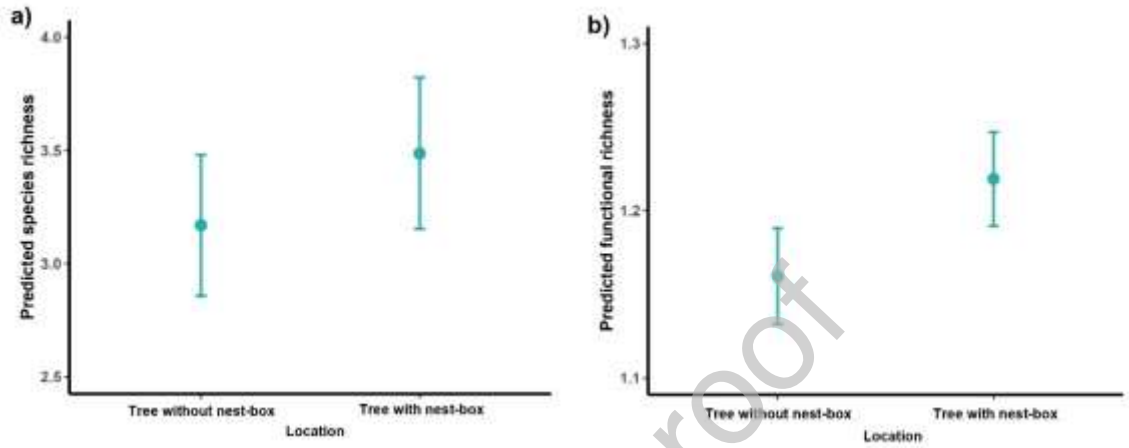
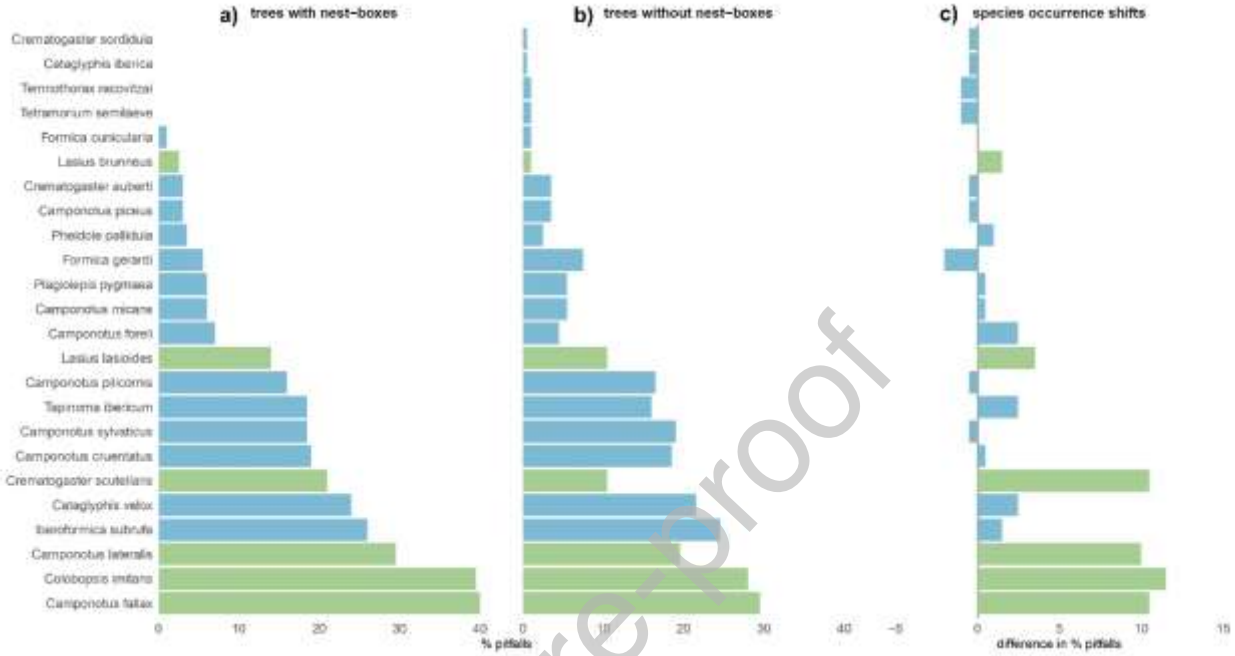


Figure 3. Occurrence of ant species (percentage of pitfall traps) in trees with nest-boxes a) and without nest-boxes b). Panel c) shows the difference in occurrence between trees with and without nest-boxes for each species. Arboreal species are shown in green, whereas non-arboreal species are shown in blue.



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: