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ORIGINAL PAPER

Road proximity and an invasive ant mediate the occurrence patterns of native ant species

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Abstract Road networks are expanding globally, altering ecosystems and facilitating the spread of invasive species. We investigated how road proximity and the invasive Argentine ant (*Linepithema humile*) influence native ant occurrence in a Mediterranean oak forest (Montado) ecosystem, a High Nature Value farmland. Ants were sampled by pitfall trapping across three distances from roads (0, 50, and 100 m) alongside the collection of local environmental measurements. We collected 3,287 ants (32 species), with *L. humile* exhibiting an incidence of 68.6%. A two-step analytical framework evaluated these species patterns. First, using univariate models, we found that the managed roadside verge (0 m) significantly altered local abiotic conditions, causing a localized spike in soil pH, an increase in understory vegetation height and plant richness, and a severe deficit in topsoil leaf litter accumulation compared to the interior forest (50 and 100 m distance from road). Second, species-specific piecewise structural equation models (pSEMs) demonstrated that these road-mediated micro-habitat modifications and the presence of *L. humile* may act as simultaneous, overlapping filters structuring some native ant species occurrence. The invader operated as a biotic filter, influencing the occurrence patterns of five of the eight analysed native focal species (*Aphaenogaster gibbosa, Crematogaster scutellaris, Plagiolepis schmitzii, Tapinoma nigerrimum, and Tetramorium semilaeve*). Our findings show that roads not only modify habitat

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conditions but may also mediate the impact of invasive species on native biodiversity.

Keywords Road ecology · Road distance effects · Ants · Argentine ant · Montado

Introduction

The expansion of road networks has been closely associated with the development of human societies, by improving human connectivity and providing access to services and markets (Laurance et al. 2014). However, the spread of the road network and traffic have also profound impacts on ecosystems and wildlife (D'Amico et al. 2026; Forman & Alexander 1998; Van der Ree et al. 2015). In particular, the physical and environmental changes associated with road presence may affect the patterns of occurrence and abundance for a variety of wildlife organisms (Benítez-López et al. 2010; Torres et al. 2016). Such road-induced changes may fragment and disrupt habitats for some species, but may also provide movement and dispersal corridors for plants and animals, including native (Ascensão et al. 2012; Kütt et al. 2016; Phillips et al. 2019; Vanneste et al. 2020; Villemey et al. 2018), and non-native species (Bergamin et al. 2022; Brown et al. 2006; Stiles & Jones 1998). Thus, road-related effects can trigger complex responses in communities, not only through direct impacts, namely road mortality, habitat alteration and fragmentation, but may also mediate the influence of invasive species on native communities (Ascensão et al. 2020, 2022).

While the bulk of research of road effects on species occurrence patterns has been dedicated to large body-sized and conservation concern species, road ecology research on small-bodied species, such as insects, has seldom been considered (Barrientos et al. 2021). Yet the impacts of roads on small and ground-dwelling insect species are likely to be particularly strong in the areas adjacent to roads. This is because the construction and operation of a road generally implies significant changes in microclimatic and soil characteristics along the road corridor, which may affect the species composition and structure of local plant communities. Likewise, the management of road verge vegetation, carried out to maintain driver visibility along the right-of-way, can significantly conditionate the vegetation composition and structure, especially within the first meters from the asphalt where regular cutting occurs (Forman & Alexander 1998; Vanneste et al. 2020). As so, the presence and maintenance of roads and their verges may alter the habitat continuum, leading to considerable changes in local biotic and abiotic conditions (Forman & Deblinger 2000), with expected significant impacts on invertebrate communities. Regarding insects, the handful of studies dedicated to road effects have reported disparate patterns, with some showing a higher diversity either close (Amatta et al. 2023; Samways et al. 1997; Skórka et al. 2018) or far from the roads (Haskell 2000), and others showing differences at the level of species' traits (Rebrina et al. 2022). Additionally, the interaction between roads, native and invasive species in shaping community dynamics remains largely understudied.

Understanding how road proximity is related to modifications in ant communities may provide valuable information for road and landscape management towards the conservation of insect biodiversity (assuming ants as bioindicators), preservation of ecosystem services, and control of invasive species. For this, we examined how road proximity may be linked to changes in environmental conditions and how these changes, in turn, affect ant community composition. Ants are a good model organism given their high abundance and occupancy, local high species richness, diverse levels of specialisation, ease of sampling and identification, and fast reactivity to environmental changes (Read & Andersen 2000; Tiede et al. 2017). Moreover, the effects of habitat changes on ant communities can be measured at relatively short spatial intervals (< 100 m). As a result, ants are frequently used as the study subject to identify environmental changes at finer spatial scales and in long-term monitoring studies (e.g., Andersen et al. 2002; Underwood & Fisher 2006; Wendt et al. 2021a). Ants also account for well-known invasive species, some of which being responsible for severe impacts on native biodiversity, ecological processes, local economies and human well-being (Angulo et al. 2022; Gruber et al. 2022; ISSG 2015; Lowe et al. 2000). In particular, the Argentine ant (*Linepithema humile*), originally from South America, is a highly successful invasive species that has spread worldwide, including Europe, Australia, South Africa and North America, thriving in urban, suburban, agricultural, and natural environments (Lowe et al. 2000; Wetterer et al. 2009). The

species' success is due to its ability to form massive supercolonies, adaptability to various environments, and its aggressive behaviour towards other ant species (Moffett 2012; Van Wilgenburg et al. 2010). This species has significant ecological and economic impacts, such as displacing native ant species, disrupting ecosystems, and damaging crops (e.g., Angulo et al. 2024; Carpintero & Reyes-López 2008).

While previous research has documented general edge effects on ant assemblages, studies simultaneously tracking how roadside environmental modifications interact with such biological invasions to impact native populations remain scarce, particularly within unique, high-value socio-ecological systems like the Mediterranean Montado. To address this gap, we implement a two-step analytical pathway framework to investigate how road proximity and *L. humile* occurrence interactively shape the population-level patterns of native focal ant species. Specifically, we test three explicit, interconnected hypotheses:

(1) Road proximity and its associated edge land management create a distinct, localized abiotic filter, significantly altering micro-habitat conditions at the immediate road verge (0 m) compared to the intact forest interior matrix (50–100 m).
(2) The invasive Argentine ant preferentially exploits these modified roadside micro-habitats, showing a positive association with altered environmental attributes characteristic of the managed road verge.
(3) Native ant species exhibit species-specific responses driven by simultaneous filters, experiencing an indirect negative biotic filter from Argentine ant occurrence (local exclusion) alongside separate, independent direct micro-habitat filtering driven by the road-altered environmental properties.

Materials and methods

Study area

The study was carried out at Companhia das Lezírias (CL), a state-owned property that includes the list of Long-Term Ecological Research (LTsER) stations in Portugal, focusing specifically on the Montado ecosystem (www.ltsermontado.pt) (Fig. 1). Montado is an agrosilvopastoral system with a savanna-like physiognomy, being dominated by evergreen oak trees (*Quercus suber* and *Q. rotundifolia*) with variable densities. It is considered a High Nature Value Farming System being managed using a sustainable multifunctional approach favouring diverse human activities, such as cork extraction, cattle grazing and agriculture (Pinto-Correia et al. 2011).

The property covers nearly 18,000 hectares and includes several habitat types, but the Montado covers an extensive area (ca. 7,000 ha). Numerous environmental and ecological studies and monitoring projects have been conducted at CL, targeting various animal and plant groups (Hipólito et al. 2016; Matos 2022; Wendt et al. 2021a). CL is surrounded by three paved roads (N10, N118 and N119) with identical characteristics (ca. 10 m wide, moderate traffic intensity, with verges being cleaned once a year). The Argentine ant has been present in Portugal since 1890 and is now widespread across the country (Dias 1955; Espadaler & Gómez 2003). It was recently found to also occur in the study area (Wendt et al. 2021a, 2021b).

Sampling design and ant identification

Ant sampling was performed in Spring of 2022, corresponding to the seasonal period of highest ant activity. Sampling was conducted along the three roads surrounding CL using pitfall traps (Fig. 1), with six replicate sites on each road. Individual transect locations were selected based on three structural criteria: (1) the presence of a sufficiently wide road verge to ensure safe field work; (2) continuous, uninterrupted Montado habitat extending from the road tarmac edge to a distance of at least 200 m; and (3) a minimum spatial separation of 500 m between adjacent sites to ensure spatial independence.

Each site comprised three parallel sampling lines deployed at fixed distances perpendicular to the road tarmac axis (Fig. 1): 0 m (the managed road verge), 50 m, and 100 m. The 50-m distance represents the immediate ecotone marking the transition into the open woodland, positioned just beyond the road verge and its mandatory fire-prevention ploughed strip, while the 100-m distance lies entirely within the stable core matrix of the Montado forest. Each sampling line consisted of a linear cluster of five pitfall traps

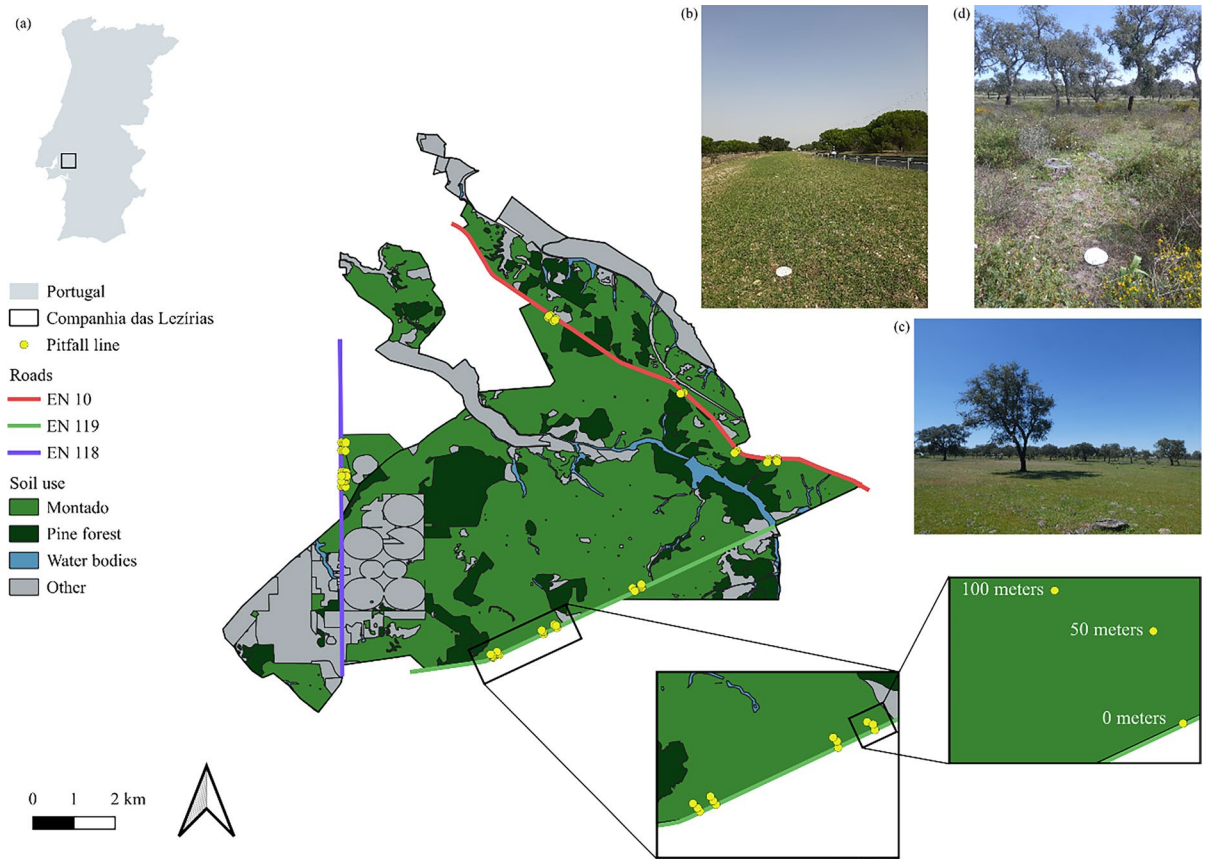

Fig. 1 **a** Location of the study area in Portugal; **b** distribution of the pitfall lines (yellow dots) near three roads bordering Companhia das Lezírias. Each sampling site includes three distances from the road: 0 m (road verge), 50 m, and 100 m into the Montado habitat

spaced 5 m apart, resulting in 54 sampling lines and 270 individual pitfall traps deployed across the study landscape (3 roads × 6 sites × 3 distances × 5 traps).

The pitfall traps were made of 250 mL rigid plastic cups with a 65 mm opening and were buried in the ground up to the rim level. The cups were filled with ethylene glycol (10%) jointly with a few drops of liquid detergent to break down the surface tension. Additionally, a cardboard plate was put on top (leaving space for epigean invertebrates to fall into the trap) to reduce unintentional vertebrate captures and avoid flooding from rain. The traps stayed in the field for 15 days, after which they were collected, and the samples were stored in 70% ethanol.

Taxonomic identification of ant specimens was performed with the help of an Olympus SZX7 stereomicroscope and using specialized literature (Arcos & García 2023; Collingwood & Prince 1998; Lebas et al. 2017). The specimens were later deposited in the laboratory of entomology at the Faculty of Sciences, University of Lisbon.

Environmental information

In our study area, previous studies showed that ant occurrence patterns are influenced by environmental factors such as soil characteristics (e.g., granulometry, organic matter content) and vegetation structure (Wendt et al. 2021a, 2021b). To disentangle the environmental filtering mechanisms driving ant occurrence patterns, local soil properties and vegetation structure were characterized at each of the 54 spatial sampling lines.

Soil information

In each pitfall line one soil sample (ca. 500 cm^3, composed by five subsamples) was collected at

approximately 5 to 20 cm soil depth near the pitfall traps (ca. 1 m) to analyse pH, organic matter content and soil granulometry. This procedure was consistent across all pitfall lines at every road distance. In the laboratory, samples were sieved using a 2 mm sieve to separate the soil from larger components such as stones, leaves, and roots. Then, approximately 5 g of air dried soil from each sample was diluted in demineralized water (1:5, v/v), and the pH value of the solution was measured using a pH meter (Hanna Instruments HI10530).

To determine the organic matter content of soil, the Loss on Ignition method was used (Heiri et al. 2001); ca. 5 g of soil per sample, dried at 60 °C, was incinerated in a Muffle furnace (Nabertherm L3/11/C6). Gradual heating up to 550 °C was conducted for two hours and maintained for 4 h. After the samples cooled down to room temperature in a desiccator, they were weighed again, with the relative difference in weight relating to the organic matter content present in the sample.

Approximately 200 g of each soil sample (< 2 mm) was processed using sequential sieves to evaluate soil granulometry, separating the fine-grained soil (silt and clay, < 75 µm) from the sand fraction (75–2000 µm). The sieves were placed in a sieve-shaker (Fritsch Analysette 3) and shaken for five minutes to separate sand from finer particles (silt and clay). The separated materials were then weighed to determine the relative proportion of soil particles.

We expected soil organic matter to increase with distance from the road because traffic-related disturbances—such as mechanical soil compaction and reduced vegetation cover—typically suppress litter accumulation and slow organic matter inputs near road edges (Forman & Alexander 1998). In contrast, we anticipated higher soil pH in areas closer to the road, as roadside environments often receive substantial deposition of alkaline dust derived from road materials and vehicle-generated particulates, which can elevate pH in adjacent soils (Farmer 1993). We also expected soil granulometry to differ near the road, with a relatively higher proportion of fine particles (silt and clay) farther from the road, as road construction, traffic and periodic verge management can increase soil disturbance and erosion near road edges, potentially promoting the removal of finer particles from exposed roadside soils (Deljouei et al. 2018).

Vegetation information

For the characterization of the plant community, three 10-m-long transects were located in each pitfall line, where traps were set up. Data on plant richness, and plant height were taken every 50 cm along the transects using the point-intersect method (Nunes et al. 2017), while also recording the percentage of ground cover with leaf litter, grasses and shrubs. Additionally, variation in plant community height between pitfall lines was described using the Community Weighted Mean (CWM) of the plant height (i.e., the mean community height weighted by species abundances (Chozas et al. 2017)). This analysis was performed using *tidyr* (Wickham et al. 2023a) and *dplyr* (Wickham et al. 2023b) packages in R.

We expected leaf litter accumulation to increase with distance from the road because tree density and canopy cover are usually high farther from road edges, leading to greater inputs of leaves and woody debris in less disturbed areas (Watkins et al. 2003). Plant species richness was also anticipated to be higher away from the road, as roadside environments experience stronger edge effects, pollution, and physical disturbance that reduce the establishment and persistence of many species (Forman & Alexander 1998). Plant height is expected to shift with road distance due to the removal of dominating upper canopy trees along managed verges, allowing fast-growing ruderal vegetation to thrive. Similarly, we expected shrub cover to increase with distance from the road, given that chronic disturbance, altered soil conditions, and mechanical damage from traffic tend to suppress shrub growth in close proximity to road verges (Trombulak & Frissell 2000).

Data analysis

Prior to analyses, we first selected a set of environmental variables from the pool of descriptors that were not highly correlated. We used the 'findCorrelation' function from the caret package (Kuhn 2008), which considers the absolute values of pairwise Pearson correlations. If two variables are highly correlated ($r > |0.7|$), the function calculates the mean absolute correlation for each and removes the variable with the larger mean absolute correlation (see Supplementary material S1). We retained seven variables: plant richness, plant height, organic matter, pH, percentage

of finer particles (silt and clay), leaf litter, and shrub cover. To stabilize model optimization and facilitate the direct comparison of standard path coefficients, all continuous environmental predictors were z-score standardized (mean = 0, SD = 1) before analysis.

For each trap line, we calculated ant species incidence (proportion of active pitfall traps within a single sampling line in which a given species is present) since this approach helps to reduce potential bias caused by the overrepresentation of certain species due to factors such as ant nest proximity (Gotelli et al. 2011).

Drivers of species occurrence

To model the cascading impacts of road networks and biological invasions under realistic sample constraints (N = 54 trap lines), we applied a two-step analytical framework. This modelling strategy prevented the overparameterization and loss of statistical degrees of freedom that occur when fitting fully saturated causal networks to field data of modest sample sizes. In the first step, we isolated the micro-habitat changes driven by infrastructure proximity using univariate one-way analyses of variance (ANOVAs), treating the continuous environmental features as independent responses and road distance (Verge [0 m], 50 m, and 100 m) as a categorical factor. Post-hoc multiple comparisons were conducted via Tukey's Honest Significant Difference (HSD) tests using the 'multcomp' package to identify exactly where structural environmental transitions occurred across the gradient. Environmental variables were advanced into subsequent modelling procedures only if they exhibited a significant directional response ($P < 0.05$) to the road proximity gradient.

In the second step, we evaluated the species-specific responses of eight focal native ant species (those detected in at least 8 trap lines) using piecewise structural equation modelling (pSEM; Shipley 2016) implemented via the piecewiseSEM package (Lefcheck 2016). Piecewise SEM accommodates localized generalized linear models (GLMs) fitting non-normal error distributions and validates network path structures via a localized d-separation framework. For each focal native species, a structural network was built by pairing two linked equations: (1) a baseline model predicting the occurrence of the invasive Argentine ant from the road-sensitive micro-habitat features, and (2) a focal model evaluating how native species occurrence is shaped by the simultaneous, additive filters of Argentine ant presence alongside direct paths from the road-sensitive environmental attributes.

All local models were specified as binary GLMs (using species occurrence—presence/absence at the trap line level) utilizing a binomial error distribution and a logit link function. To explicitly account for localized trap disturbances caused by wild boars during the sampling period, the number of active, intact pitfalls recovered per line was incorporated as an explicit offset term in every GLM, standardizing varying trapping intensities. Automated backward stepwise variable selection based on the Akaike Information Criterion (stepAIC function, MASS package) (Akaike 1974; Johnson & Omland 2004) was deployed within each local equation to remove uninformative environmental predictors and maintain network optimization. Global model goodness-of-fit was verified via directed separation tests to check for missing causal paths (independence claims), generating Fisher's C statistics where a global P-value > 0.05 indicated an adequate non-parsimonious model fit. Explanatory power for each node was assessed using Nagelkerke's pseudo-R^2. Path-level direct, indirect, and total effects were extracted using the semEff package (Murphy 2023), with 95% confidence intervals generated via 100 bootstrap runs. All data restructuring and statistical analyses were executed in R version 4.3.2 using the 'tidyr' (Wickham et al. 2023a) and 'dplyr' (Wickham et al. 2023b) packages.

Results

Overall ant occurrence patterns

A total of 3,287 individual ants were collected, representing 32 species (see Supplementary Material S2). The Argentine ant dominated the assemblage with 1,900 individuals (57.8% of total abundance), followed by *Messor barbarus* (280 individuals, 8.5%) and *Tapinoma nigerrimum* (173 individuals, 5.3%). The Argentine ant occurred in 68.6% of all pitfall lines. Several native species, including *Aphaenogaster iberica*, *Camponotus fallax*, *Formica gerardi*, *Goniomma hispanicum*, *Messor lusitanicus*, and *Proceratium melinum*, occurred in low abundance (< 10

individuals) with distributions restricted to fewer than five pitfall lines.

Environmental shifts

Univariate analyses of variance (ANOVAs) revealed distinct, parameter-specific habitat and soil structure variations across road distance (Table 1 and Fig. 2). Soil pH demonstrated high sensitivity to road proximity ($F_{2,48}=12.445$, $P<0.001$), with more basic/alkaline conditions recorded at the immediate road verge compared to the interior forest matrix. Post-hoc Tukey HSD tests confirmed that soil pH neutralized rapidly away from the road, with significant decreases at 50 m ($P<0.001$) and 100 m ($P<0.001$), while the two interior stations remained statistically homogeneous ($P=0.810$). Understory vegetation height community-weighted means also varied significantly across the distance from the road ($F_{2,48}=8.764$, $P<0.001$), driven by elevated vegetation heights along the managed road verge compared to the interior spaces (Tukey HSD: Verge vs. 50 m, $P=0.002$; Verge vs. 100 m, $P=0.001$), with no discrepancy between 50 and 100 m ($P=0.971$). The depth of the accumulated leaf litter layer (Leaf litter) increased progressively away from the road infrastructure ($F_{2,48}=7.649$, $P=0.001$), displaying a significant accumulation deficit at the verge compared to the core forest habitat at 100 m ($P<0.001$). Understory vascular plant species richness (Plant richness) was similarly altered across the gradient ($F_{2,48}=3.443$, $P=0.040$), reflecting an accumulation of ruderal and weed species at the verge compared to the 100 m matrix ($P=0.031$).

Conversely, understory shrub cover ($P=0.379$), soil organic matter ($P=0.591$), and finer particles texturing ($P=0.938$) exhibited stable, flat distributions across the distance stations. Based on these macro-gradient filters, only the four road-sensitive attributes showing significant variation (Soil pH, Height cwm, Leaf litter, and Plant richness) were advanced into the subsequent causal path analyses.

Species-specific piecewise structural equation models

The baseline model predicting the occurrence of the invasive Argentine ant was optimized using two road-sensitive habitat variables selected via stepAIC: HEIGHT and LITTER (Table 2). The model revealed that Argentine ant occurrence was strongly and positively associated with the taller plant height structures characteristic of the managed road verges. Conversely, accumulated leaf litter depth showed only a weak, non-significant positive trend (Table 2).

The species-specific piecewise structural equation models for the eight focal native ant species achieved good overall goodness-of-fit metrics, with all Fisher's C test *P*-values exceeding 0.05, validating the structural path configurations (Table 2 and Fig. 3). The presence of the Argentine ant emerged as a severe biotic filter, exerting strong, highly significant negative associations with five of the eight analyzed native species. The strongest signal was detected for the *Tapinoma nigerrimum*, where the interactive pressure of invasive exclusion alongside an operational affinity for more basic soils accounted for half of its total occurrence variance (Table 2 and Fig. 3). Highly significant exclusion filters by the invader were similarly confirmed for other four species: *Plagiolepis schmitzii*, *Aphaenogaster gibbosa*, *Crematogaster scutellaris*, and *Tetramorium semilaeve* (Table 2 and Fig. 3).

Table 1 Summary of one-way ANOVA and Tukey HSD post-hoc test results evaluating the effects of road distance (Verge [0 m] vs. 50 m vs. 100 m) on habitat and soil attributes ($DF_{Distance}=2$, $DF_{Residuals}=48$ for all models)

Environmental variable	Code	*F*-statistic	*P*-value	Post-hoc Pattern (Tukey HSD)
Soil pH	PH	12.445	< 0.001	Verge > 50 m = 100 m
Understory vegetation height community-weighted means	HEIGHT	8.764	< 0.001	Verge > 50 m = 100 m
Leaf litter	LITTER	7.649	0.001	100 m > Verge; 50 m = Verge
Plant species richness	RICHNESS	3.443	0.04	Verge = 50 m; Verge > 100 m
Shrub cover	SHRUB	0.99	0.379	Verge = 50 m = 100 m
Topsoil organic matter	ORGANIC	0.532	0.591	Verge = 50 m = 100 m
Finer particles texturing	SILT	0.064	0.938	Verge = 50 m = 100 m

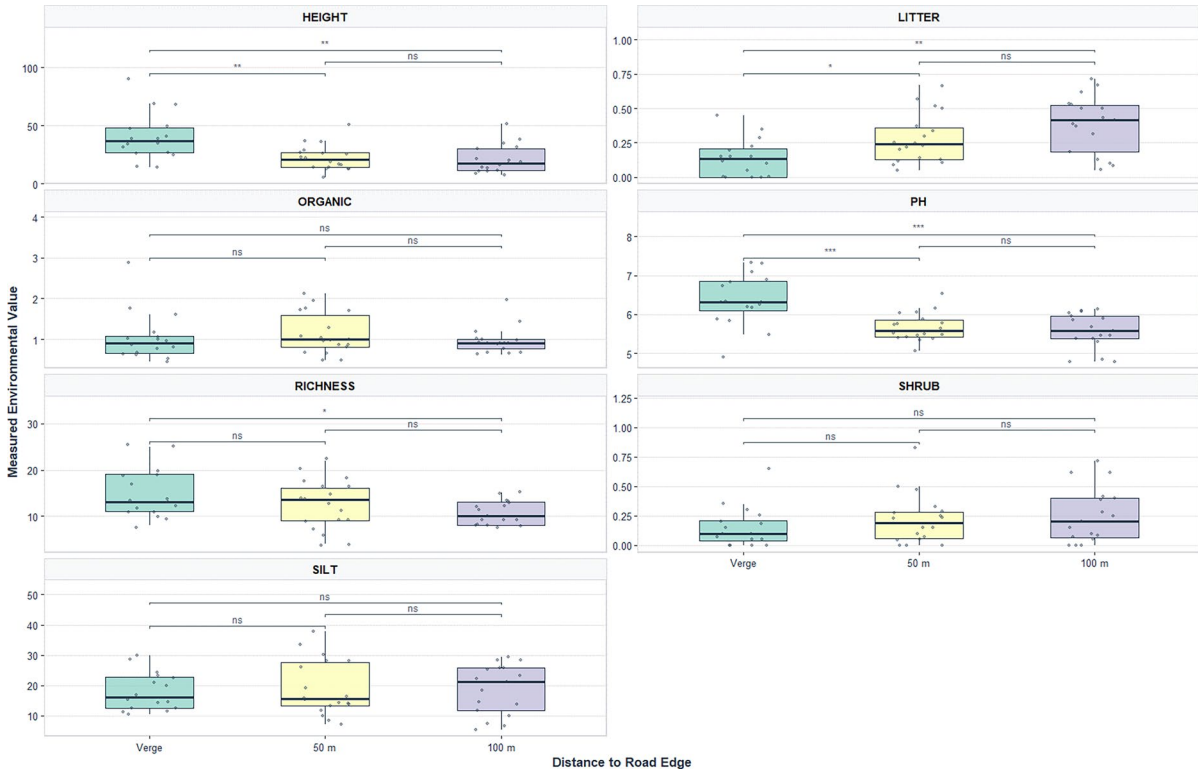

Fig. 2 Spatial relationships between local micro-environmental characteristics and distance to the road edge (Verge, 50 m, and 100 m) across the Montado ecosystem matrix. Panels are organized alphabetically by uppercase variable codes: vegetation understory height (HEIGHT), topsoil accumulated leaf litter depth (LITTER), topsoil organic matter content (ORGANIC), topsoil pH (PH), vascular plant species richness (RICHNESS), finer particles texture classification (SILT), and understory shrub canopy cover (SHRUB). Boxplots illustrate median values and interquartile ranges (IQR), with raw data points overlaid to show full distribution density. Pairwise statistical significance levels derived from post-hoc Tukey HSD tests are displayed via stacked comparison brackets (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; ns, non-significant)

Concurrently, local micro-habitat modifications driven by road proximity also conditioned native species distributions, independently of the invader's presence. Taller understory vegetation structures exerted complex, individualistic constraints across the species, with a direct positive trend for *Tetramorium semilaeve*, and negative directional trends for *Tapinoma erraticum* and *Messor barbarus* (Table 2 and Fig. 3). Finally, localized soil modifications directly filtered the populations of *Temnothorax recedens*, with a negative association with increasingly basic soils close to the road network (Table 2 and Fig. 3).

Discussion

We aimed to examine the impact of roads on local ant communities by assessing the effects of road distance in soil and vegetation conditions and its influence on the occurrence of the invasive Argentine ant. We hypothesized that the Argentine ant would thrive in road verges because road construction and management alter local habitat conditions (e.g. microclimate, soil, vegetation), further affecting the occurrence of native ant species and paving the way for its establishment. We found that road proximity was responsible

Table 2 Path coefficients and model fit statistics evaluating environmental and associations with ant occurrence. For the invasive Argentine ant (*Linepithema humile*), parameter estimates are derived from an optimized baseline generalized linear model (GLM); for all native focal species, estimates are derived from piecewise Structural Equation Models (pSEMs) wherein the baseline *L. humile* model was integrated as a standard component of the structural pathway network. Fisher's C statistics are reported to reflect overall pSEM global goodness-of-fit

Focal species	Predictor node	Estimate (β)	Std. error	*P*-value	Global fit fisher's C (*P* value)	Response R^2
Linepithema humile	HEIGHT	1.408	0.658	0.033	*NA*	0.15
	LITTER	0.713	0.469	0.128		
Tetramorium semilaeve	*Linepithema humile*	− 1.371	0.688	0.046	2.29 (0.318)	0.14
	HEIGHT	0.527	0.319	0.098		
Tapinoma erraticum	HEIGHT	− 0.647	0.384	0.092	5.62 (0.230)	0.09
Crematogaster scutellaris	*Linepithema humile*	− 1.452	0.711	0.041	3.09 (0.543)	0.13
Messor barbarus	HEIGHT	− 1.103	0.622	0.076	9.11 (0.167)	0.2
	PH	0.608	0.375	0.105		
Temnothorax recedens	PH	− 0.883	0.472	0.062	13.89 (0.085)	0.14
Plagiolepis schmitzii	*Linepithema humile*	− 2.744	0.895	0.002	4.70 (0.319)	0.35
Aphaenogaster gibbosa	*Linepithema humile*	− 2.347	0.951	0.014	4.13 (0.659)	0.35
	RICHNESS	− 0.925	0.531	0.081		
Tapinoma nigerrimum	*Linepithema humile*	− 3.454	1.22	0.005	5.40 (0.494)	0.5
	PH	0.839	0.441	0.057		

for significant changes on specific soil and vegetation characteristics (e.g. plant richness, plant height, soil pH and leaf litter cover; Table 1) of the Montado ecosystem, thus supporting previous studies which showed that roads may strongly influence local abiotic and biotic conditions (Munyati & Menwe 2018; Zhou et al 2020). In our study area, road maintenance works are responsible for maintaining a low presence of trees and tall shrubs along road verges, thus leading to low soil cover by leaf litter. However, these regular works also seem to favour the occurrence of several disturbance-tolerant plant species (Forman & Deblinger 2000; Vanneste et al. 2020), which grow along road verges and are rarely found within the Montado matrix. The combined effect of road operation and maintenance leads to small scale changes on local soil, microclimate and vegetation characteristics which often have consequences on the composition and structure of animal communities, particularly invertebrates (Canelo et al. 2024; Rae et al. 2006; Wu et al. 2023).

The Argentine ant was found to be widespread in the study area exhibiting a high overall occupancy. However, contrary to our initial expectations, it achieved a high dominance across spatial gradients extending into distances farther from the road (see Supplementary Material Table S2). The habitat plasticity of this invasive species coupled with its tolerance to human-driven disturbance (contrary to most native species) may have allowed it to expand across the entire area, enabling it to dominate resources both near and far from road verges (Roura-Pascual et al. 2010; Van Wilgenburg et al. 2010). In fact, these ants seem to benefit from the taller vegetation near roads which may host larger populations of prey and mutualistic hemipterans that provide them with nutrient-rich honeydew (Banerjee et al. 2024). Importantly, the positive association between *L. humile* occurrence and plant height should not be interpreted as evidence that the Argentine ant is restricted to, or occurs predominantly at, the road verge. Rather, our results suggest that road-associated habitat modification may locally increase habitat suitability for *L. humile*, while the species itself is already broadly distributed across the Montado matrix. Plant height was significantly higher at the road verge than at 50 and 100 m, and *L. humile* occurrence was positively associated with this environmental gradient. However, contrary to our expectations, this invasive ant was also dominant at distances further from the road suggesting that it is well-established in the study area, forming extensive colonies. Thus, the road effect appears to operate

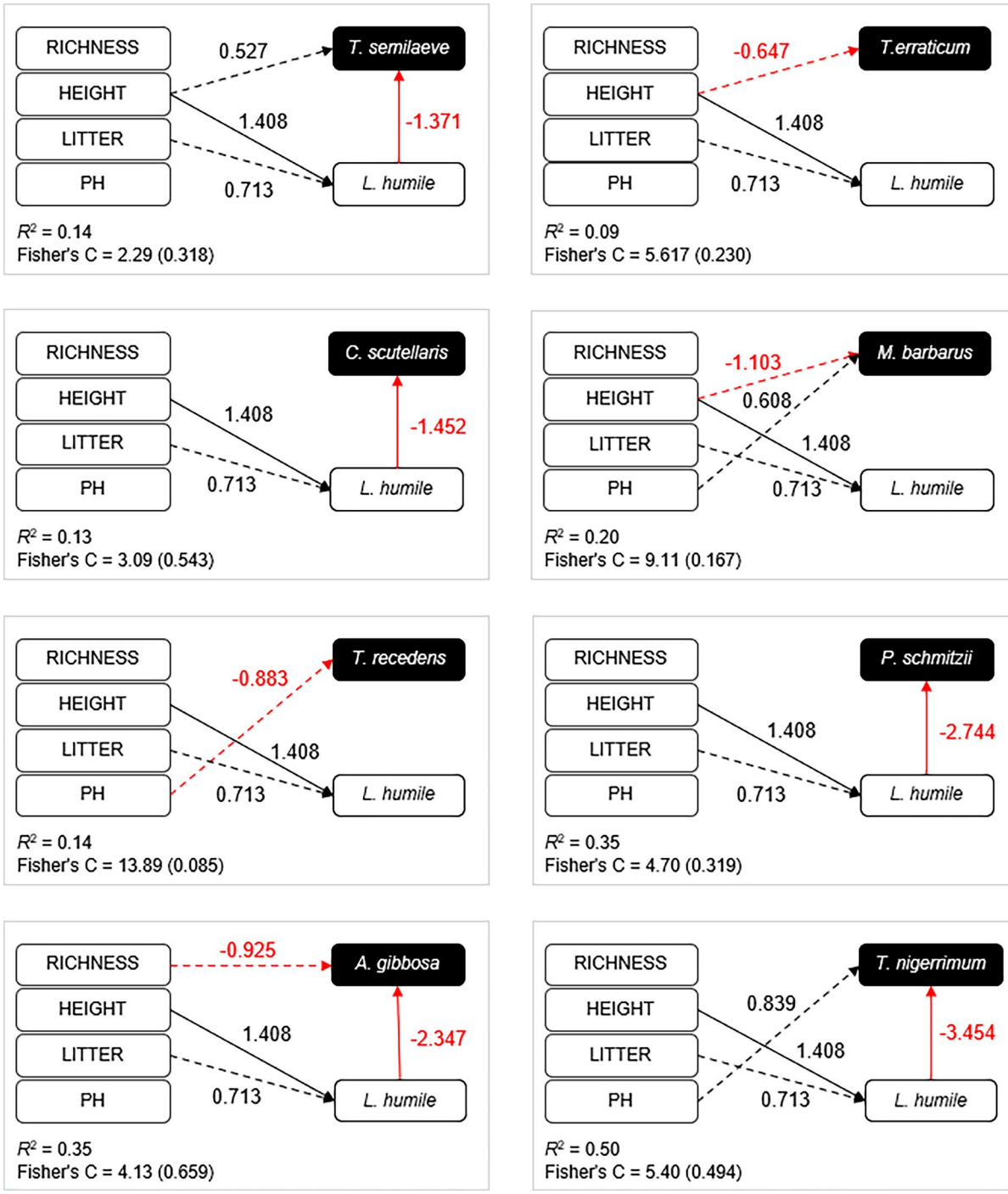

Fig. 3 Results of piecewise structural equation models (SEMs) showing the combined effects of retained predictors plant richness (RICHNESS), plant height (HEIGHT), leaf litter (LITTER), and soil pH (PH), together with the presence of Argentine ant (*L. humile*) on the occurrence of native ant species. Red arrows indicate negative relationships, while black arrows indicate positive relationships. Dashed lines indicate weaker effect ($p > 0.05$). R^2 values represent the proportion of variance explained. Fisher's C and corresponding *P*-value between brackets are reported

primarily through modification of local habitat conditions rather than through a simple spatial concentration of *L. humile* at the road edge. Previous studies carried out on Mediterranean oak forests have shown the high invasiveness of this ant species, how they benefit from human disturbance to establish and spread, and their lasting negative effects on these ecosystems affecting both native species richness and ecological processes (Angulo et al. 2024; Carpintero et al. 2003; Roura-Pascual et al. 2010). Other studies have also reported severe impacts of the Argentine ant on local ant and invertebrate communities, direct and indirect effects on vertebrates, and the disruption of important ecological processes, like pollination and myrmecochory (Boieiro et al. 2018; Holway 1999; Naughton et al. 2020; Sanders et al. 2003; Wendt et al. 2021a; 2022).

Our findings showed that the Argentine ant influences the occurrence of several native ant species, likely leading to their displacement from the invaded areas. In our study, the occurrence patterns of five native ant species (*A. gibbosa, C. scutellaris, P. schmitzii*, *T. nigerrimum*, and *T. semilaeve*) were influenced by the presence of the Argentine ant. All of these species (but *A. gibbosa*) are considered generalists that feed opportunistically on invertebrate prey as scavengers and using other seasonally available resources (Roig & Espadaler 2010). Also, several ant species which are common in Montado (*Aphaenogaster senilis*, *Camponotus* spp., *Cataglyphis* spp.) were found in very low abundance or seem to be absent in the study area, most probably as a consequence of the invasion by the Argentine ant. These species face a strong competition for resources and space from the Argentine ant in invaded areas, being often displaced from their nests in highly infested areas due to the aggressive behaviour and mass recruitment of the invasive ant (Carpintero & Reyes-López 2008; Holway 1999; Roura-Pascual et al. 2010). However, coexistence is possible at lower densities of the invasive species, particularly for submissive native ants and those with specific ecology and behaviour (e.g. some *Temnothorax* spp.), which have low contact with the invasive species due to spatial and temporal segregation of their activity (DiGirolamo 2006; Gippet et al. 2022; Roura-Pascual et al. 2010).

We found evidence of direct effects of road proximity on the occurrence of several native ant species due to changes in soil and vegetation conditions, suggesting that native species are balancing habitat preferences with constraints imposed by both the invasive Argentine ant and road proximity. For example, the granivorous harvester ant *M. barbarus* is known to prefer open and hot habitats and may benefit from the vegetation clearing carried out in road verges (Azcárate & Peco 2003; Roig & Espadaler 2010). Our results indicate that the distribution of native species, which is primarily shaped by micro-environmental variables at local scales—including those altered by road proximity—is further modulated by the presence of the Argentine ant. Indeed, native ants are known to adjust their spatial patterns in response to fine-scale environmental variations as well as in response to the presence of the Argentine ant in edge areas (Bolger 2007; Wendt et al. 2021c).

Nevertheless, several study limitations must be acknowledged. The road verges examined here are narrow and structurally simplified, making them unlikely to sustain stable, continuous strips of taller vegetation capable of providing adequate shelter, microclimatic buffering, or suitable foraging opportunities for disturbance-sensitive native species. Although our measured predictors captured a substantial portion of the observed variation, additional unmeasured variables or stochastic processes likely contribute to the detected species patterns. In particular, key microclimatic conditions, such as shading, canopy closure, and soil moisture availability, covary with our environmental predictors but were not directly quantified due to the absence of continuous, in-situ dataloggers, representing an inherent constraint of the study. Additionally, shrub cover in this system is shaped by two overlapping and sometimes counteracting drivers: localized road-edge disturbance and broader agrosilvopastoral management practices characteristic of Montado properties, such as livestock grazing and periodic understory clearing. Because these land-use actions intersect our sampling layout, they can override or mask local road-edge patterns, explaining the absence of a consistent linear distance signal for this variable. Road traffic further introduces unmeasured pressures, including noise, physical vibrations, and chemical pollutants that can alter soil and vegetation chemistry. Together, these traffic-related stressors and the structural simplification of road edges may reinforce the dominance of invasive species while intensifying the competitive

exclusion of native fauna, ultimately accelerating biodiversity loss and weakening ecosystem stability.

Conclusions

In conclusion, roads are known to impact local biodiversity, but their effects can be quite severe when they simultaneously favour the occurrence and spread of invasive species. The impact of the Argentine ant together with road effects can lead to the extinction of local populations of vulnerable ant species and a deterioration in ecosystem services provided by native ants, such as seed dispersal, pollination, or biological control (Folgarait 1998; Gómez & Oliveras 2003; Wagner et al. 2004; Wendt et al. 2022; Wills & Landis 2018).

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Declarations

Conflicts of interest The authors declare no conflict of interests.

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