UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

Does road proximity influence the structure and composition of ant communities?

Tomás da Silva Pinto

Mestrado em Biologia da Conservação

Dissertação orientada por:
Doutor Fernando Ascensão
Doutor Mário Boieiro

2023

Acknowledgements

Chegada a hora de terminar esta etapa resta-me agradecer a toda a gente que fez parte dela.

Ao Fernando, que teve a ideia original de trabalhar com formigas e que, se não fosse ele não estava atualmente a evangelizar a palavra mirmecológica a toda a gente. Balizou-me, orientou-me e transmitiu-me o conhecimento do que é ser um Biólogo.

Ao Mário, a minha fonte de conhecimento e de gosto pelas formigas, por toda a ajuda desde o início até ao final. Ajudou-me a compreender a importância e a curiosidade que um bicho tão pequeno pode ter!

Ao Eng. Rui Alves e à Sandra Alcobia da Companhia das Lezírias e ainda ao Sr. José Palha por permitirem e encorajarem a realização deste trabalho nos seus terrenos.

Ao Sergio Chozas e à Helena Serrano pelas preciosas ajudas na área das plantas e do solo, respetivamente.

A todos os que me ajudaram no campo naqueles dias longos a cavar buracos ou a tirar copos do chão. Martim, Dávid e Rafael, obrigado!

Uma palavra de apreço especial para o Tiago, o Tomás, a Raquel e o Gonçalo não só pela ajuda no campo como por todo o companheirismo, amizade e amor que me têm demonstrado nos últimos e particularmente neste ano.

Um gigante e especial obrigado à companheira de vida, Marta, por ter estado lá do início ao fim, surfando os altos e baixos do costume sempre a puxar por mim para conseguir atingir os meus objetivos. Obrigado por tudo, amo-te.

À minha família por estar lá sempre que eu precisei. Em especial aos meus avós por serem uma inspiração para mim, ao meu irmão por me apoiar em tudo e aos meus pais por serem a razão pela qual estou aqui hoje, como sou, a escrever isto para todos vós.

Obrigado, Natureza, por seres tão bela.

Abstract

The number of roads has been continuously increasing, which in turn increases their impacts on the ecosystems. These infrastructures present several advantages for human activities. However, they also impact natural ecosystems and consequently local animal communities, such as invertebrates. Ants (Hymenoptera: Formicidae) are an example of a group of invertebrates that is significantly impacted by local disturbances like roads. Due to their ecological traits, such as high abundance and species richness, as well as their fast response to environmental changes, ants are frequently used as bioindicators. This study aims to assess how roads in the Montado system affect ant communities. Pitfall traps were used to collect ants on three separate roads at three different distances from the road (0, 50, and 100 meters), in three seasons (Autumn, Winter, and Spring). The ant community displayed no discernible response to road distance, although some species exhibited marked responses to road proximity. The invasive Argentine ant (*Linepithema humile*) was detected in high numbers in many sampled sites. The presence of this species was negatively correlated with the native ant fauna. The results suggest a road effect on a species level rather than on the ant community. The presence of the Argentine ant was favoured by cattle grazing and poses a great threat to the native fauna. This invasive species can inflict severe consequences in the ecosystem as some ecosystem services provided by native species may collapse. Future research should focus on disentangling the impact of invasive species on the road effect on local invertebrate communities.

Keywords: Road ecology, Bioindicator, Ants, Argentine ant, Montado

Resumo alargado

A constante expansão das estradas a nível global promove vários benefícios para as sociedades humanas, no entanto acarreta muitas desvantagens, nomeadamente no que concerne os ecossistemas circundantes. A construção de uma estrada num ambiente natural provoca uma série de efeitos negativos para a vida selvagem tanto diretos (mortes causadas por colisões com veículos ou perda de habitat) como indiretos (efeito barreira ou poluição). No entanto, as estradas também criam corredores ecológicos que podem ser essenciais para a conexão de diferentes populações de uma dada espécie. Todavia, estes corredores são muitas vezes aproveitados por espécies invasoras para a sua dispersão. O impacto das estradas em vertebrados é relativamente bem conhecido, não acontecendo o mesmo para os invertebrados. Dado o pequeno tamanho de alguns invertebrados é difícil a compreensão do impacto direto das estradas nestes animais, havendo alguns estudos sobre o efeito barreira das estradas nestas comunidades. Alguns estudos ditam as estradas como infraestruturas prejudiciais para certos grupos, outros estudos demonstram um aumento de diversidade de insetos perto das estradas. As formigas (Hymenoptera: Formicidae), são um grupo de invertebrados muitas vezes utilizado como bioindicador ambiental dadas algumas das suas características (elevada abundância e diversidade, exploração de diferentes recursos, facilidade de amostragem e identificação, rápida reação a alterações ambientais, entre outras). As estradas conseguem alterar condições a uma escala fina tanto no solo, como nas condições ambientais, provocando alterações de abundância nas espécies presentes. No entanto, ainda não se sabe como estas infraestruturas alteram a estrutura e composição de algumas comunidades de invertebrados, nomeadamente de formigas. Neste sentido, o objetivo deste estudo é compreender como as estradas influenciam a estrutura e composição das comunidades de formigas. Para isso, foram amostradas as comunidades de formigas a diferentes distâncias de três estradas que circundam a Companhia das Lezírias (N10, N118, N119). Em cada estrada foram montadas armadilhas do tipo pitfall a três distâncias da estrada (0, 50 e 100 metros). Em cada distância foram montadas cinco armadilhas ao longo de um transeto paralelo à estrada. Cada estrada teve três replicados (distanciados a mais de 100 metros cada), contando assim com 135 armadilhas no total (3 estradas x 3 transetos x 5 pitfalls x 3 replicados). As armadilhas consistiram em copos de plástico rígido de 250 mL com uma abertura de 65 mm enterrados no solo e cheios com etilenoglicol. Todas as armadilhas foram montadas em zonas de Montado. A amostragem foi efetuada no Outono, Inverno e Primavera. Na Primavera os replicados foram duplicados (6 por estrada, totalizando 270 armadilhas). Os indivíduos capturados foram depois levados para laboratório e identificados com base em literatura especializada. Foi feita uma caracterização ambiental dos locais de estudo com base em variáveis consideradas importantes na estruturação das comunidades de formigas. Foram recolhidas variáveis relacionadas com o solo (pH, matéria orgânica e composição de areias), plantas (altura e riqueza) e ainda a cobertura da canópia das árvores. Para a análise dos resultados foram calculados os três principais números de Hill (qD) de modo a obter uma informação geral da comunidade. Estes números correspondem à riqueza de espécies ($q = 0$), ao índice de diversidade de Shannon-Wiener ($q = 1$) e ao índice de diversidade de Simpson ($q = 2$). Em seguida, e para avaliar a resposta das comunidades à distância da estrada, foi estudada a relação entre as comunidades de formigas usando a técnica de Non-metric Multi-Dimensional Scaling (NMDS). Devido ao elevado número de variáveis ambientais recolhidas foi conduzida uma Análise de Componentes Principais (PCA), de modo a reduzir a dimensionalidade dos fatores ambientais para posteriormente incluir nos modelos. A distribuição das dez espécies com maior ocorrência foi modelada com Modelos Generalizados Lineares Mistos (GLMMs) onde foram incorporados os dados de presença/ausência de cada espécie, os dados ambientais (as duas ou três primeiras componentes da PCA), a distância da estrada e ainda a presença da formiga-argentina, uma espécies invasora que foi detetada em elevada abundância em muitos locais de amostragem. Foram capturados 6416 indivíduos pertencentes a 35 espécies/morfoespécies, sendo a formiga-argentina, *Linepithema humile*, a mais representada nestes dados com 4603 indivíduos (72%) seguida da *Messor barbarus* (205 indivíduos; 3%).

Foram ainda capturadas algumas espécies mais raras, como *Hypoponera eduardi* ou *Proceratium melinum*. A última foi o primeiro registo desta espécie para Portugal, tendo sido encontrada perto da estrada N118. A estrada N119 foi a que teve maior abundância (4460 indivíduos), enquanto a N10 foi a estrada com maior riqueza específica (27 espécies). No que diz respeito à distribuição ao longo das várias distâncias foi possível verificar que os valores de abundância e riqueza mais elevados foram registados a 50 metros da estrada (2925 indivíduos e 27 espécies). Os índices de diversidade calculados demonstraram valores mais elevados de riqueza, Shannon e Simpson para a estrada N10 comparada com as outras duas. Os valores foram comparados e as diferenças encontradas entre estradas foram significativas, no entanto, as diferenças entre distâncias não. Os dados de NMDS revelaram uma resposta mais clara da comunidade à estrada N10 face às diferentes distâncias. Ainda assim, os GLMMs calculados demonstraram diferentes respostas das diferentes espécies face à estrada. Espécies como *Tapinoma erraticum* e *Solenopsis* sp. aumentaram a sua probabilidade de ocorrência com a distância à estrada. Outras espécies (*Temnothorax* sp., *Crematogaster scutellaris* e *Tapinoma nigerrimum*) tiveram uma tendência oposta. Os modelos comprovaram ainda o impacto da formiga-argentina, uma vez que a probabilidade de ocorrência das espécies nativas estava negativamente correlacionada com a presença da espécie invasora. Os fatores ambientais considerados também tiveram efeito na distribuição das espécies, com diferentes espécies a ter diferentes respostas aos mesmos fatores ambientais. Os resultados obtidos demonstraram que a comunidade de formigas não sofre alterações significativas na sua composição e estrutura com a distância da estrada. No entanto, parece haver uma resposta de algumas espécies. Diferentes espécies apresentaram diferentes distribuições face à estrada tendo em conta fatores ambientais e a presença da formiga-argentina. A espécie invasora restringiu a distribuição de quase todas as espécies nativas dada a sua agressividade e dominância. A presença desta espécie invasora deveu-se principalmente ao desenvolvimento de atividades de pastoreio presentes no local de amostragem. O pisoteio promovido pelo pastoreio é uma fonte de perturbação para o habitat das formigas que acaba por homogeneizar o ambiente e, assim, promover o estabelecimento de espécies mais resistentes à perturbação, como é o caso da formiga-argentina. O efeito do pastoreio foi ainda o motivo para a comunidade de formigas ser distinta na estrada N10, uma vez que nestes locais não existe pastoreio e a vegetação tem uma composição mais estruturada e complexa face aos outros locais mais impactados. Apesar disto, espécies com uma distribuição semelhante revelaram respostas opostas aos mesmos fatores ambientais, revelando assim haver uma resposta a uma microescala baseada em cada espécie individualmente. Concluindo, a gestão das bermas das estradas assume um papel crucial na conservação destes corredores ecológicos que são muitas vezes aproveitados por espécies não-nativas. Deverá ser ainda adotada uma gestão sustentável do gado, através da criação de zonas naturais sem gado, dado o seu impacto nas comunidades locais de invertebrados. Estas zonas irão funcionar como reservatórios para populações fragmentadas de espécies afetadas pela homogeneização da vegetação e do solo. A formiga-argentina apresenta uma ameaça para o ecossistema através da remoção de certos serviços de ecossistema promovidos pelas espécies nativas pelo que se deverá avaliar melhor os seus impactos e promover o seu controlo em áreas mais sensíveis. Estudos futuros deverão investigar como as estradas impactam as comunidades de invertebrados, enquanto isolam o impacto das espécies invasoras do efeito da estrada.

Palavras-chave: Ecologia das estradas, Bioindicador, Formigas, Formiga-argentina, Montado

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1. Introduction

The expansion of road networks has been closely associated with the growth of human societies. Nonetheless, despite the many advantages of road networks (i.e., transport routes, connectivity), an increase in the amount of road area and automobile traffic frequently influences nearby natural ecosystems.

The construction and proliferation of roads have emerged as a pivotal concern in modern environmental discourse due to their profound negative impacts on ecosystems and wildlife. Roads fragment and disrupt habitats, leading to habitat loss, isolation, and alter resource availability for a variety of wildlife species. Moreover, roads amplify mortality rates through collisions with vehicles and hinder essential movement patterns, effectively constraining genetic diversity and impeding species' ability to adapt to changing environments (van der Ree et al., 2015; Ascensão et al., 2017).

Furthermore, the development of these transportation routes leads to issues regarding water runoff, namely a rise in floods (IBRD, 2016; Zemke, 2016), and sediment deposition (Bian and Zhu, 2009). Given that they encourage the dispersion of contaminants from both water and sediment runoff, these effects become more significant (Herngren et al., 2006; Tromp et al., 2012). These contaminants, notably heavy metals such as lead, mostly harm the plants that absorb them (Raskin et al., 1994; Neher et al., 2013). Further ecological effects might include habitat fragmentation, the barrier effect that encourages genetic impoverishment (Bhattacharya et al., 2003; Keller and Largiadèr, 2003; Rico et al., 2007; Ascensão et al., 2016), as well as all the pollution (noise, air, soil, and visual) that comes with the usage of these roads (Scanlon, 1987; Rydell, 1992; Lengagne, 2008).

Despite its barrier effect, roads also frequently serve as ecological corridors that facilitate animal dispersal and mobility; nevertheless, this phenomenon appears to favour a variety of species that can withstand disturbance, including some exotic species, some of which are invasive (Stiles and Jones, 1998; Brown et al., 2006). Roadsides are ideal habitats for more generalist plant and animal species (Ascensão et al., 2012). Roadside clearance promotes the establishment and growth of disturbance-tolerant plants (Tyser and Worley, 1992; Angold, 1997; Mortensen et al., 2009; Joly et al., 2011), changing the typical structure and composition of the plant community. Roadside management frequently entails the planting of woody plants, which can be invasive and spread into neighbouring systems (Richard and Deblinger, 2000). Hence, for certain local populations, roadways, and especially their verges, show both beneficial and harmful traits.

Road encroachment can impact communities and ecological processes. It's important to understand its effects for road and landscape management, alongside biodiversity conservation. While it’s known roads affect certain species (D'Amico et al., 2016; Cooke et al., 2020), there's a gap in knowledge regarding the effects on community composition.

Following previous research on vertebrates (Ascensão et al., 2022), it was questioned whether road encroachment leads to significant changes in communities of insects. In comparison to vertebrates, the research on how roads affect insect communities is much less developed. Because of their small size, dead insects are difficult to detect on roads, therefore little is known about the mortality brought on by vehicle impacts. Yet, flying insects are known to have great fatality rate on roads (Martin et al., 2018). The barrier effect of roads, which was previously mentioned, seems to impact more flying than soildwelling insects (Mader et al., 1990; Pfister et al., 1997). Because insects are highly susceptible to contaminants in the air or soil (as are all invertebrates in general), road traffic pollution affects them as well (Gate et al., 1995; Ryalls et al., 2022).

Depending on the taxonomic groups being studied, the type of the road (such as its width and traffic volume), and the surrounding environment (more open or more closed environments), road effects on insects can vary significantly. Some studies show a higher species richness of insects close to the road (Samways et al., 1997; Skórka et al., 2018), whereas many others show the exact opposite (Haskell, 2000; Carpio et al., 2009).

Due to their traits, ants (Hymenoptera: Formicidae) are a group of invertebrates frequently used as bioindicators (Majer, 1983). These traits include their extreme abundance, high specific richness, many species' specialisations, occupancy of higher trophic levels, ease of sampling and identification, and reactivity to environmental changes. As a result, ants are frequently used as the study subject to identify environmental changes at a finer spatial scale and are used in long-term monitoring studies (Underwood and Fisher, 2006). Furthermore, ants provide several types of services such as regulating services (Del Toro et al., 2012). Regulating services may include seed dispersal (Lengyel et al., 2010), pollination (Gómez and Zamora, 1992) or biological control (Van Mele, 2008). Due to these (and many more) services, these tiny insects can have large-scale impacts in terrestrial ecosystems, hence being called "ecosystem engineers".

Recent research has demonstrated the value of this insect group for responding to various land uses (Andersen et al., 2002; Chen et al., 2011; Frasconi Wendt et al., 2021a), for rehabilitating mining sites (Majer, 1992; Ribas et al., 2012), and for restoring forest yields (Schmidt et al., 2013; Staab et al., 2014). Studies that examine the effects of roads on diverse systems also utilise ants. Several of this research concentrate primarily on how important roadside ecological value is for ant diversity (Samways et al., 1997; Tshiguvho et al., 1999; Vieira-Neto et al., 2016). Nevertheless, there aren't many studies in the field that examine how ant communities respond to distance from roads.

Overall, roads are likely to cause changes in the soil and micro-climatic conditions, which can lead to changes in the local occurrence and abundance of different species, and in turn in the functioning of the ecosystem and their services. However, there is still a great lack of knowledge regarding the way roads may lead to changes in insect communities, particularly in ants.

In this way, the fundamental goal of this dissertation is to test if road proximity is associated with changes in the composition and structure of local ant communities. The underlying hypothesis is that ant communities far from roadways are different relative to communities inhabiting road vicinity since a richer and more abundant ant community is expected far away from the road, with a less impacted environment from the different road disturbances.

2. Methods

2.1 Study area

The study was carried out at Companhia das Lezírias (38º 500' N, 8º 490' W), a state property on the list of LTsER (Long Term Ecological Research) Montado research and monitoring stations in Portugal. This property, which has a total land size of 17,952 hectares, is an agrosilvopastoral system and includes several habitat types, but the cork oak forest covers an extensive area (6,751 ha) (www.ltsermontado.pt). Numerous environmental and ecological studies and monitoring projects have been conducted on these lands with an emphasis on various animal and plant species (Hipólito et al., 2016; Frasconi Wendt et al., 2021a; Matos, 2022). This property is surrounded by three paved roads (N10, N118 and N119) with identical characteristics (ca. 10 m wide, traffic intensity mainly composed by trucks and cars and road verges with the same maintenance).

2.2 Ant sampling

Ant sampling was performed along the three roads, N10, N118 and N119. Three transects were established parallel to each road at different distances from the road at 0, 50 and 100 meters (Figure 2.1). In each transect, five pitfall traps were set at five-meter intervals. Each road had three replicates (three sets of the three distances), totalling 135 traps (3 roads x 3 transects x 3 replicates x 5 pitfalls) (see Table 6.1 in Supplementary material). All replicates were set in Montado areas, at least 100 meters from each other. The 0 meter distance is considered the road verge, the 50 meter distance is the start of the Montado (beyond the road verge and the ploughed strip obligatory for fire prevention), and the 100 meter distance is entirely within the Montado patch. The start of the Montado was recognized as the area where the vegetation changes to a typical Montado vegetation structure.

The pitfalls 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 liquid detergent to break down the surface tension and radiator antifreeze liquid (10% ethylene glycol) to preserve the collected specimens. 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 stored in 70% alcohol. Sampling was performed in Autumn, Winter, and Spring. In Spring, the number of replicates in each road was doubled (a total of 270 pitfall traps) as it was the season with the highest ant activity as well as when the environmental variables were collected.

2.3 Species identification and classification in functional groups

An initial sorting of the specimens was done in the laboratory, followed by the taxonomic determination of the ants. The identification work was completed with the help of an Olympus SZX7 stereomicroscope and specialized literature (Collingwood and Prince, 1998; Seifert, 2002, 2012; Galkowski and Wegnez, 2017). The specimens sampled were deposited in the entomology laboratory at the Faculty of Sciences, University of Lisbon.

A disturbance-response classification was used to group similar species in terms of their ecological role in the ecosystem. This classification follows the work of Roig and Espadaler (2010) that was made for the Iberian ant fauna. The functional groups identified in this study are the following: Cryptic (C), Generalists/Opportunists (GO), Invasive/Exotic (IE), Cold Climate and/or Shadow Habitat

Specialists (CCS/SH), Hot Climate and/or Open Habitat Specialists (HCS/OH) and Specialist Predators (SP).

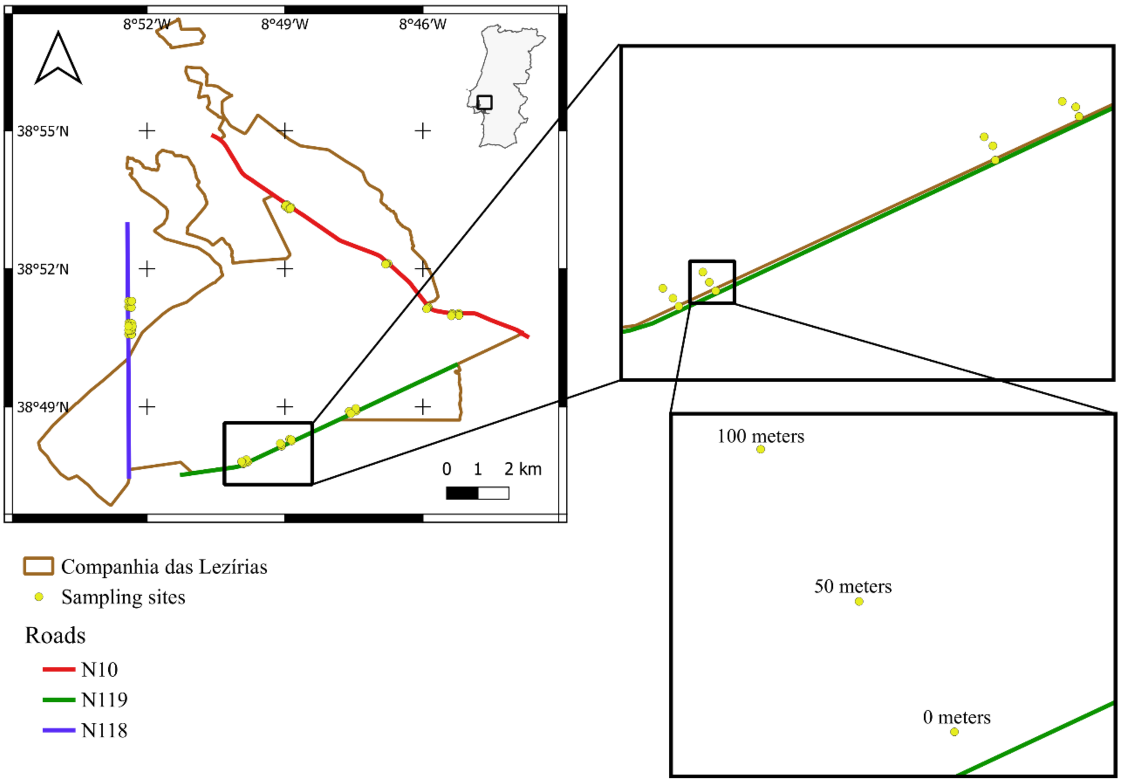

Figure 2.1 - Sampling sites' location in and near Companhia das Lezírias, Portugal. The three roads sampled are coloured and in each transect (set of three sampling sites) each dot represents one sampling site, corresponding to one distance (0, 50 and 100 meters) from the road.

2.4 **Collection of environmental information and variables**

A set of environmental variables known to influence ant communities was recorded to control for environmental variability (see Table 6.2 in Supplementary material). Ant communities are known to be driven by environmental factors such as vegetation structure, soil characteristics (e.g., granulometry, organic matter content), and temperature (Frasconi Wendt et al., 2021a, 2021b). Accordingly, at each sampling site, it was collected information on soil, plants, and canopy cover. All environmental variables were recorded in the Spring when most ant species peak their activity, and because it is the best season to identify most plant species.

2.4.1 Soil

One soil sample was collected per sample site (distance per transect) at the depth where the traps were set up (ca. 10 cm) to determine soil type, organic matter, and pH, totalling 54 samples of ca. 500 mL each. Each sample was sieved with a 2 mm sieve opening to separate the soil from larger components such as stones, leaves, or roots. Then, approximately 5g of soil per sample was taken, diluted in demineralised water (1:5, v/v), and the pH value of the solution was obtained using a pH meter (Hanna Instruments HI10530). To determine the organic matter content the Loss on Ignition method was used (Heiri et al., 2001), ca. 5 g of soil per sample, dried at 60ºC, was placed in pre-dried porcelain cups, and placed in a Muffle furnace (Nabertherm L3/11/C6). Gradual heating up to 550°C was conducted for two hours and this temperature was maintained for 4 hours. After the equipment reached a safe temperature, the samples were removed and weighed again, with the difference in weight relating to the organic matter present in the sample. Additionally, about 200 g of each sample was placed on two sequential sieves with openings of 75 and 125 µm to evaluate soil granulometry. The sieves were placed in a sieve-shaker (Fritsch analysette 3) for five minutes at speed three, to separate the sands from finer particles (silt and clay). The contents were then collected and weighted to determine the relative soil particles composition.

2.4.2 Plants

For the characterization of the local flora and vegetation, in each sampling location, three 10-meter-long transects were performed whenever possible to assess plant species richness. Also, plant heights were measured every 50 centimetres along the same transect using the point-count transect method (Nunes et al., 2014).

Functional diversity indices, namely functional richness, evenness, dispersion, and Rao's quadratic entropy (Villéger et al., 2008), were calculated using the *FD* R package (Laliberté et al., 2014). These indices were all calculated only using height as trait. Additionally, the Community Weighted Mean (CWM) of the plant height (i.e., the community plant height value weighted by the abundance of species in the community) was assessed using *tidyr* (Wickham et al., 2023a) and *dplyr* (Wickham et al., 2023b) R packages.

2.4.3 Canopy cover

A photograph using a mobile phone (Samsung S23) facing upwards was taken at each trap site at the same height and with the same camera opening. The photographs were then analysed using ImageJ software (Schneider et al., 2012). In this software, each image was binarized, separating the pixels corresponding to the tree canopy with one value from the pixels of the sky with another. The canopy cover at each site was obtained by calculating the percentage of pixels corresponding to the tree canopy compared to the total number of pixels. The bigger the area covered by the canopy, the lower the soil temperature and the greater the number of leaves that will fall (Thompson and Eldridge, 2005). Hence, canopy cover may influence other factors, such as soil temperature and leaf litter, that usually influence ant diversity and activity.

2.5 **Data analysis**

2.5.1 Ant diversity, abundance, and incidence

The study of ant communities followed the framework proposed by Chao et al. (2014) based on Hill numbers (^{q}D) (Hill, 1973). This framework is commonly used in biodiversity studies since Hill numbers provide complementary information using the following formula:

$$^{q}D = \left(\sum_{i=1}^{R} p_i^{q} \right)^{1/(1-q)}$$

Three Hill numbers were calculated, namely species richness ($q = 0$), Shannon-Wiener diversity index ($q = 1$) and Simpson's diversity index ($q = 2$). These metrics were calculated for each road, season, and road distance. They were compared using the Kruskal-Wallis test. In the case of significance, Dunn's post hoc test was used to assess the differences between pairs of roads, seasons, or road distances.

Ant abundance data was heterogeneous, particularly due to the high abundances of the Argentine ant (*Linepithema humile*) (see Results) and was log-transformed. This transformation aims to reduce data skewness to approximately fit to normality. It was also determined the incidence data, meaning the number of pitfalls in each site where a given species is present. This type of data allows us to overcome the effect of species records being dominated by a single or few species e.g., due to nest proximity to a given pitfall trap (Gotelli et al., 2011).

2.5.2 Road distance effect on ant communities

To determine if road distance would be related to ant community dissimilarity, a Non-metric Multi-Dimensional Scaling (NMDS) was calculated for species and for functional groups, using both abundance and incidence data across seasons. NMDS data was used to calculate the pairwise PERMANOVA test for comparing the roads and distances.

2.5.3 Drivers of ant community structure and composition

Generalised Linear Mixed Models (GLMMs) were conducted to model the relation of occurrence with the environmental and road information. It was only modelled the occurrence of the 10 most frequent ant species as other species were recorded in few sites. Given the high number of environmental variables collected, a Principal Component Analysis (PCA) was applied to summarize the variation found in environmental variables. The first three axes (PCA1, PCA2 and PCA3) were retained for further analyses, which retained ca. 67% of the explained variance. The presence/absence of the Argentine ant was included in the models as this species is known to affect the occurrence and abundance of native ant species (Frasconi Wendt et al., 2021a). The Road variable was used as a random factor for each model.

For each native species, it was compared three nested GLMMs(m):

$$\mathrm{PA} \sim \mathrm{PCA}_{\mathrm{env}} + (1 \mid \mathrm{Road}) \qquad (m_1)$$
$$\mathrm{PA} \sim \mathrm{PCA}_{\mathrm{env}} + \text{Presence of } \textit{L. humile} + (1 \mid \mathrm{Road}) \qquad (m_2)$$
$$\mathrm{PA} \sim \mathrm{PCA}_{\mathrm{env}} + \text{Presence of } \textit{L. humile} + \text{Road distance} + (1 \mid \mathrm{Road}) \qquad (m_3)$$

where PA corresponds to the species occurrence (presence/absence) and $\mathrm{PCA}_{\mathrm{env}}$ to the PCA axes used.

To determine the best model for each species, the Akaike Information Criterion (AIC) was calculated (Akaike, 1974; Johnson and Omland, 2004) and the model with the lowest value was chosen. It was also modelled the distribution of the Argentine ant with the addition of the fourth PCA component, PCA4. The formula used on each model is presented in Table 7 (see section Results).

A correlation analysis was conducted between the Argentine ant presence and the diversity indexes of local ant communities (calculated without the invasive ant data) to test for an association between the variables. For this, it was used the Spearman correlation coefficient, which is a non-parametric measure of correlation that assesses the monotonic relationship between two variables, making it suitable for analysing non-linear associations or data with outliers (Zar, 2005). The correlation was then tested for significance using the Spearman correlation test.

3. Results

3.1. Overall patterns

A total of 6416 individuals were collected, pertaining to 35 species/morphospecies (Table 3.1). For 466 specimens, the identification was only possible at the genus level. The Argentine ant (*Linepithema humile*) was the most represented, with 4603 individuals (72%), followed by *Messor barbarus* (205 individuals; 3%), and *Temnothorax* sp. (202 individuals; 3%). Some species, like *Hypoponera eduardi* or *Formica gerardi*, were only captured once. The ant *Proceratium melinum* was for the first time recorded for Portugal (Pinto et al., 2023), found near the N118 road (at approximately 50 meters) (see Figure 6.1 in Supplementary material).

A total of 46 pitfall traps were destroyed (inactive traps range 3.3 to 46.7%, per road and distance in each season). Winter was the only season with no traps destroyed. According to the ground's evidence, most traps were destroyed by wild boars. Table 6.3 (see Supplementary material) summarises the distribution of the traps destroyed on each road at different distances.

Table 3.1 – List of species/morphospecies captured, their abundance at different road distances (in meters) and the functional group (FG) they belong, following Roig and Espadaler (2010): Cryptic (C), Cold Climate and/or Shadow Habitat Specialists (CCS/SH), Generalists/Opportunists (GO), Hot Climate and/or Open Habitat Specialists (HCS/OH), Specialist Predators (SP) and Invasive/Exotic (IE).

		Road distance (m)		
FG	**Species**	**0**	**50**	**100**
C	*Aphaenogaster gibbosa*	6	11	23
	Stenamma debile	0	38	0
	Solenopsis sp.	1	17	34
	Temnothorax sp.	30	56	116
		37	**122**	**173**
CCS/SH	*Camponotus fallax*	1	0	1
	Colobopsis imitans	0	1	8
		1	**1**	**9**
GO	*Aphaenogaster iberica*	3	4	1
	Aphaenogaster senilis	19	1	1
	Cardiocondyla elegans	25	0	0
	Crematogaster auberti	28	62	9
	Crematogaster scutellaris	49	9	21
	Myrmica sp.	0	27	0
	Pheidole pallidula	13	61	94
	Plagiolepis pygmaea	2	3	37
	Plagiolepis schmitzii	6	41	24
	Plagiolepis sp.	1	0	0
	Tapinoma erraticum	30	65	58
	Tapinoma nigerrimum	157	5	29
	Tapinoma sp.	2	3	3
	Tetramorium sp.	39	48	77
		374	**329**	**354**
HCS/OH	*Camponotus piceus*	0	0	3
	Camponotus pilicornis	0	11	5

FG	Species	Road distance (m)		
		0	**50**	**100**
	Camponotus sp.	0	1	0
	Cataglyphis iberica	0	7	2
	Formica gerardi	0	0	1
	Goniomma sp.	0	1	2
	Messor barbarus	179	19	7
	Messor capitatus	19	0	1
	Messor lusitanicus	55	30	4
	Messor marocanus	0	3	1
	Messor sp.	0	5	3
	Oxyopomyrmex saulcyi	0	42	9
		253	**119**	**38**
SP	*Hypoponera eduardi*	0	1	0
	Proceratium melinum	0	2	0
		0	**3**	**0**
IE	*Linepithema humile*	**475**	**2351**	**1777**
	Total	**1140**	**2925**	**2351**

The highest ant abundance was found on road N119 (4460 individuals), followed by N10 (1388 individuals) and N118 (568 individuals) (Table 3.2). Road N10 had the highest specifies richness values (27 species), followed by N118 (17 species) and N119 (14 species).

The 50-meter distance from the road had most ants, totalling 2925, followed by the 100-meter distance, with 2351, and the 0-meter distance, with 1140 (Tables 3.1 and 3.2). Regarding species richness, the results at the three distances were more similar than they were for species abundance; the highest value was at 50 meters, with 27 species, followed by 100 meters with 25 species, and 0 meters with 19 species.

Table 3.2. Total ant abundance per road and distance

	Road		
Distance	N10	N118	N119
0 m	597	197	346
50 m	413	136	2376
100 m	378	235	1738
Total	**1388**	**568**	**4460**

3.2. Diversity indexes

Species richness peaked at 50 meters on road N10 (26 species) and had the lowest value at 0 meters on road N118 (9 species). The Shannon index showed a higher species diversity on road N10, with the highest value at 50 meters (2.629), whereas the lowest is on road N119 at 100 meters (0.164). Simpson index results showed that there´s a higher species dominance on road N119 with the lowest value (0.052) and the highest on road N10 at 50 meters (0.910) (Table 3.3).

Table 3.3 - Ant biodiversity data (using Species richness, Shannon and Simpson index) per distance in each road across all seasons sampled.

Site	Species richness	Shannon index	Simpson index
Road N10			
0 m	22	2.138	0.821
50 m	26	2.629	0.910
100 m	22	2.250	0.842
Road N118			
0 m	9	0.426	0.167
50 m	15	1.497	0.650
100 m	18	2.047	0.831
Road N119			
0 m	11	0.715	0.286
50 m	14	0.255	0.087
100 m	10	0.164	0.052

Road N10 differs from the others regarding diversity values (Figure 3.1). This road showed higher diversity results for all three metrics used, thus containing a more diverse and equitable ant community. On one hand, species richness was not significantly different among the three roads (Kruskal-Wallis, $p > 0.05$). On the other hand, the Shannon index was different between N10 and the remaining roads (Dunn's test, N10-N118, $p=0.021$; N10-N119, $p=0.0017$). The Simpson index followed the same trend (Dunn's test, N10-N118, $p=0.014$; N10-N119, $p=0.0014$).

Temporal analysis of diversity showed only differences in species richness across seasons (Figure 3.1). Autumn and Spring were both different from Winter (Dunn's test, $p=0.020$, $p=0.0018$, respectively) but not from each other.

Road distances did not show any differences in the diversity indexes calculated (Figure 3.1).

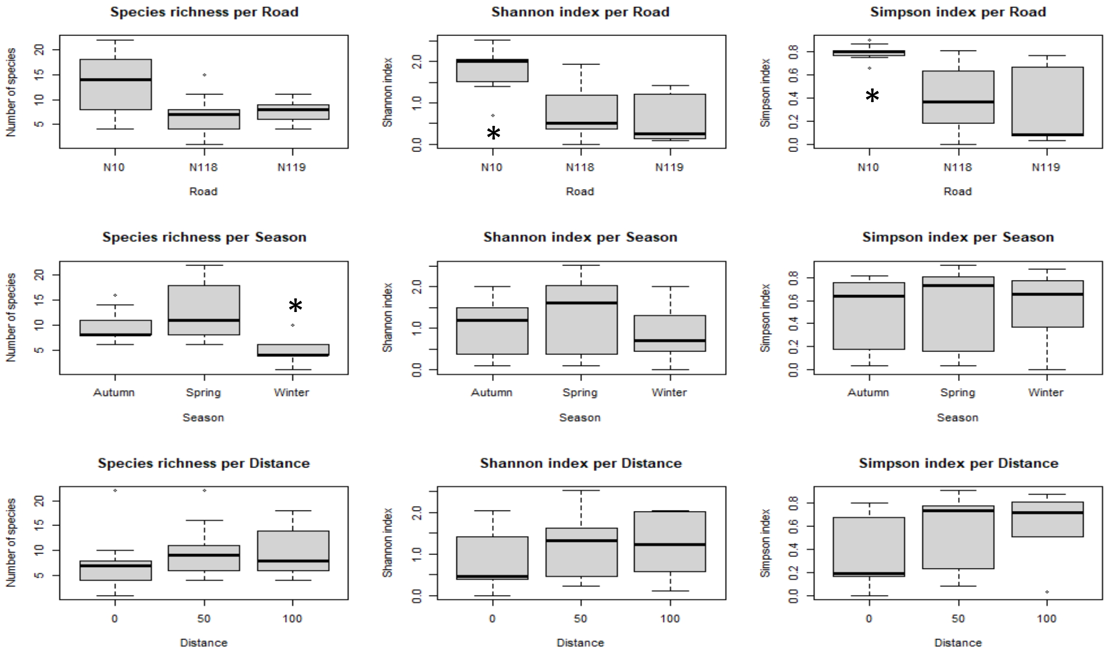

Figure 3.2 - Biodiversity indexes (Species richness, Shannon, and Simpson indexes) per road, season, and distance. Significant values ($p < 0.05$) are marked with '*'.

3.3. **Road distance effect on ant communities**

The results of the NMDS analysis (Figures 3.2 and 3.3) suggest that there is no strong evidence that road distance is related to differences in community dissimilarity (PERMANOVA $p > 0.05$). The ant communities in the closest proximity to the road were not significantly different from those in the farthest distance (Kruskal-Wallis $p=0.086$).

Both Species and Functional groups analysis did not find a relation between distance and the abundance and incidence (Figures 3.2 and 3.3). Nevertheless, road N10 revealed a distinct pattern across all NMDS graphs, evidencing the ant community's response to the road (PERMANOVA $p < 0.05$).

The Pairwise PERMANOVA showed that in Autumn and Spring (and for Winter's Functional groups' incidence) road N10 showed significant differences from the other two (N118 and N119) while these two showed no differences between each other (Table 3.4).

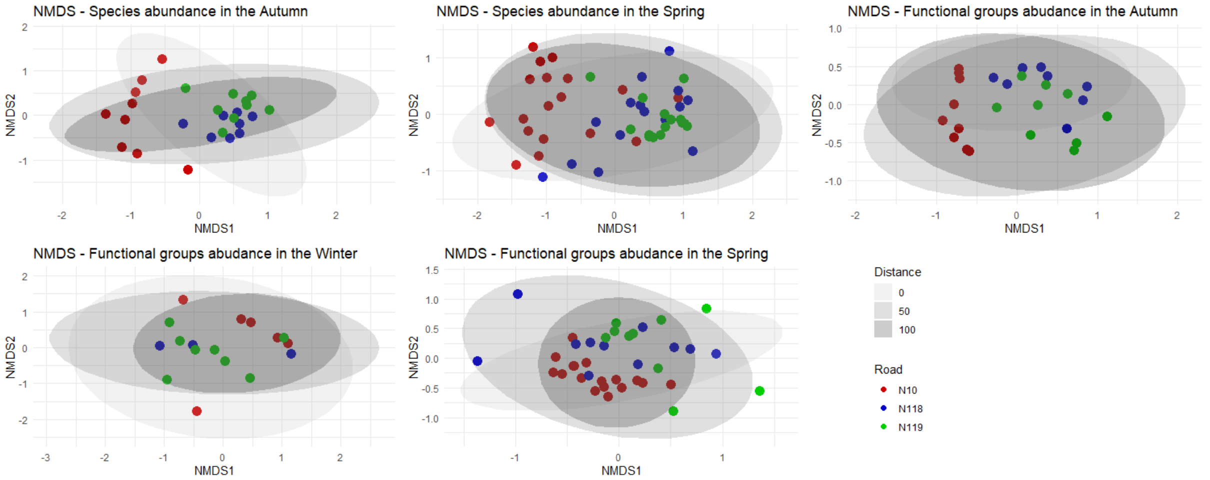

Figure 3.3 - NMDS of Species and Functional groups' abundance per season. The species abundance in the Winter NMDS is not shown as it was not computed due to the lack of data (low abundance in this season).

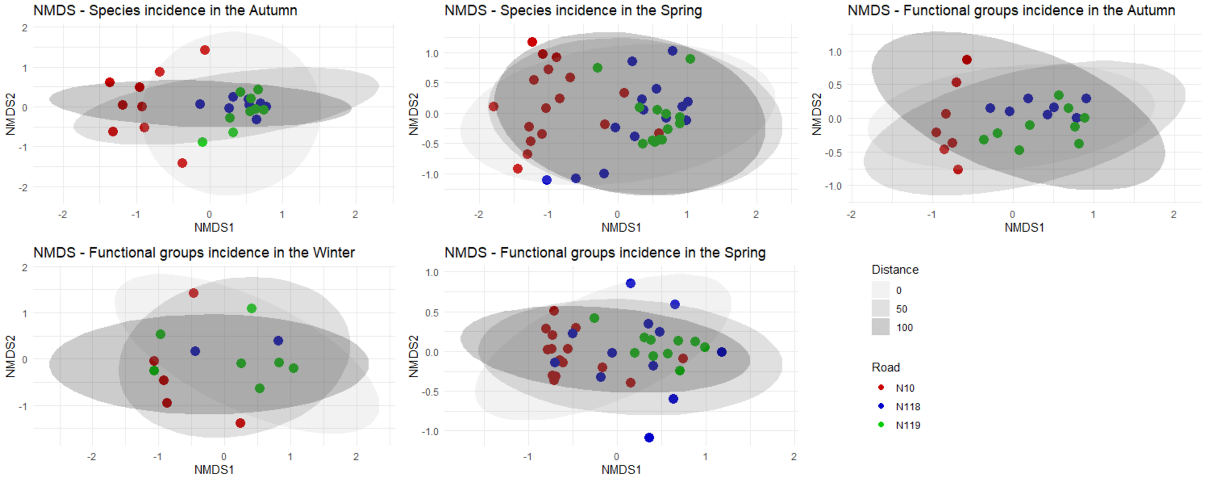

Figure 3.4 - NMDS of Species and Functional groups' incidence per season. The species incidence in the Winter NMDS is not shown as it was not computed due to the lack of data (low abundance in this season).

Table 3.4 – Differences in the abundance and incidence of ant species and functional groups between seasons and roads using Pairwise PERMANOVA tests. Significant values (p < 0.05) are highlighted.

Species Abundance

	Season		
Pairwise Permanova	Autumn	Winter	Spring
N10 vs 118	< **0.01**	-	< **0.01**
N10 vs N119	< **0.01**	-	< **0.01**
N118 vs N119	0.054	-	0.702

Functional groups Abundance

	Season		
Pairwise Permanova	Autumn	Winter	Spring
N10 vs 118	< **0.01**	0.198	< **0.01**
N10 vs N119	< **0.01**	0.375	< **0.01**
N118 vs N119	0.438	1	0.879

Species Incidence

	Season		
Pairwise Permanova	Autumn	Winter	Spring
N10 vs 118	< **0.01**	-	< **0.01**
N10 vs N119	< **0.01**	-	< **0.01**
N118 vs N119	1	-	0.750

Functional groups Incidence

	Season		
Pairwise Permanova	Autumn	Winter	Spring
N10 vs 118	< **0.01**	< **0.01**	< **0.01**
N10 vs N119	< **0.01**	0.357	< **0.01**
N118 vs N119	1	1	1

3.4. Road distance effect on functional groups

Ant functional groups showed different distribution patterns over road distances across the seasons sampled (Figure 3.4).

In Autumn, Generalist ants (GO) peaked at 50 meters since both 0 and 100 meters had similar abundances (64 and 63 individuals). The Cryptic ants group (C) increased in abundance with distance from the road (3, 29 and 51 individuals). Invasive ants group (IE) was more abundant far from the road (1602 individuals at 50 meters and 952 individuals at 100 meters) compared to the 101 individuals present at 0 meters.

The Winter revealed different distributions of these groups. The IE appeared nearer the road (31 individuals) rather than far from it (7 individuals at 50 meters and 10 at 100 meters). The other groups sampled in this season, C, GO and HCS/OH, showed a similar pattern. All these groups increased their abundance with distance from the road (0, 2 and 12; 2, 7 and 27; 1, 2 and 10, respectively).

Finally, in the Spring the relative abundance of the native groups was higher. IE increased with distance (343, 742 and 815 individuals). GO had similar abundances across the three distances (308, 213 and 265 individuals). On one hand, HCS/OH decreased with distance (251, 75 and 23 individuals). On the other hand, C demonstrated the opposite pattern (34, 91 and 110 individuals).

SP and CCS/SH groups were poorly represented. SP had 3 individuals at 50 meters, 2 in the Spring and one in Autumn. CCS/SH had 11 individuals, 1 at 0, 1 at 50 and 9 at 100 meters, all of these in the Spring.

Regarding incidence data, the overall distribution patterns are similar. IE was the group with the highest incidence followed by GO. The last one increased its incidence with road distance in the Winter. The C group followed the same trends as well, with an increase with road distance across all seasons.

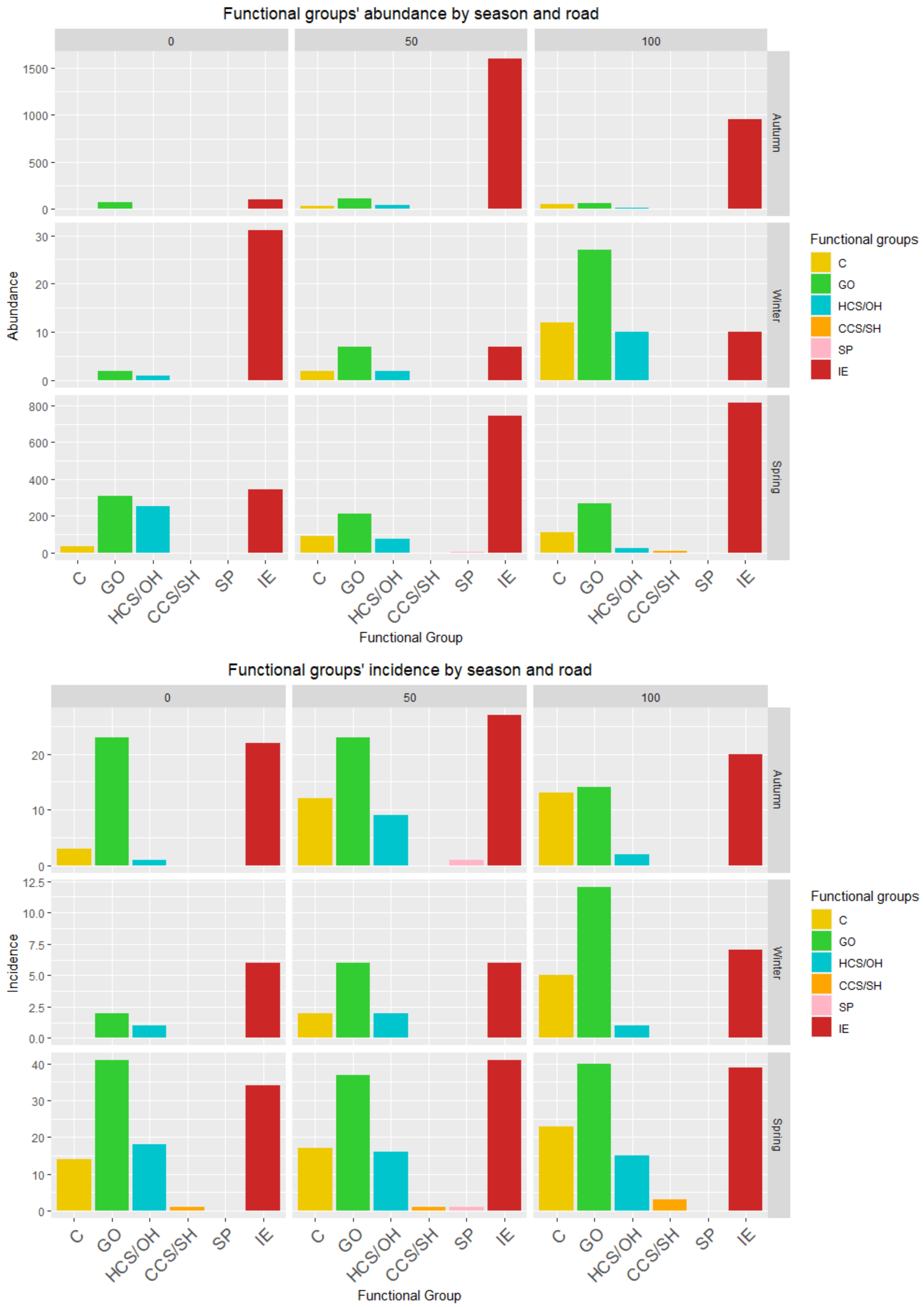

Figure 3.5 - Functional groups' (Cryptic (C), Generalists/Opportunists (GO), Invasive/Exotic (IE), Cold Climate and/or Shadow Habitat Specialists (CCS/SH), Hot Climate and/or Open Habitat Specialists (HCS/OH) and Specialist Predators (SP)) abundance and incidence by season and distance.

3.5. **Species-level analysis**

The GLMMs conducted for the ant species with the highest occurrence (see Figures 6.2 to 6.11 in Supplementary material) showed different trends in road distance effect (Table 3.5). Using occurrence data, *Linepithema humile* increases its presence with road distance with marginal significance for the larger distance (p ~ 0.06). For the other ant species, this response changed. *Tetramorium* sp. has the lowest probability of occurring at 50 meters (p < 0.001) and at the 100 meter distance with an intermediate probability between the three distances (p < 0.05). *Tapinoma erraticum* increased its presence with road distance (p < 0.05 for 100 m). *Temnothorax* sp. and *Crematogaster scutellaris* showed an opposite trend, with the highest probability of occurrence at 0 m (p < 0.05). *Messor barbarus* decreases its presence with road distance but not significantly (p > 0.05). *Plagiolepis schmitzii* has the highest probability of occurrence at 50 meters (p < 0.05). *Solenopsis* sp. increases its occurrence with road distance (p < 0.05 for 100 meters). *Aphaenogaster gibbosa* didn’t show any significant pattern (p > 0.05). At last, *Tapinoma nigerrimum* decreases its occurrence with road distance (p < 0.05 for 100 meters).

Table 3.5 - Road distance effects on invasive and native ants using GLMM. The values are in comparison to the 0 meter distance. Significant values (p < 0.05) are highlighted.

	50m		100m	
	Estimate	P-value	Estimate	P-value
Linepithema humile	0.68	0.27	1.28	0.06
Tetramorium sp.	**-2.18**	**< 0.01**	**-1.05**	**0.03**
Tapinoma erraticum	0.61	0.24	**1.20**	**0.03**
Temnothorax sp.	**-1.43**	**0.01**	**-1.52**	**0.01**
Crematogaster scutellaris	**-1.61**	**< 0.01**	**-2.18**	**< 0.01**
Messor barbarus	-0.14	0.84	-1.43	0.06
Plagiolepis schmitzii	**2.32**	**0.01**	1.88	0.04
Solenopsis sp.	2.01	0.11	**3.77**	**< 0.01**
Aphaenogaster gibbosa	0.43	0.52	-1.30	0.12
Tapinoma nigerrimum	-0.87	0.27	**-2.62**	**0.01**

3.6. **Drivers of ant community structure and composition**

As mentioned before, the invasive Argentine ant was detected in several sampling sites with high abundance and incidence values. The presence of this species seems to have a negative impact on native ant species as shown by the GLMMs results (Table 3.6). The occurrence of five ant species (*Tetramorium* sp., *Plagiolepis schmitzii*, *Solenopsis* sp., *Aphaenogaster gibbosa* and *Tapinoma nigerrimum*) were negatively impacted by the Argentine ant (p < 0.05). Other species, such as *Tapinoma erraticum*, *Temnothorax* sp., *Crematogaster scutellaris*, and *Messor barbarus* were not impacted by this invasive species (p > 0.05), hence the invasive species was not considered in the model. All these results are summarised in Table 3.6.

The correlations between the Argentine ant presence and the biodiversity indexes (calculated without the invasive ant) were almost all negative (-0.647 for abundance, 0.007 for the Shannon index, -0.171 for the Simpson index, 0.070 for ant richness and -0.529 for functional groups richness). However, only the correlations between the presence of the Argentine ant and native ant abundance and functional groups’ richness were significant (p < 0.05) (Figure 3.6).

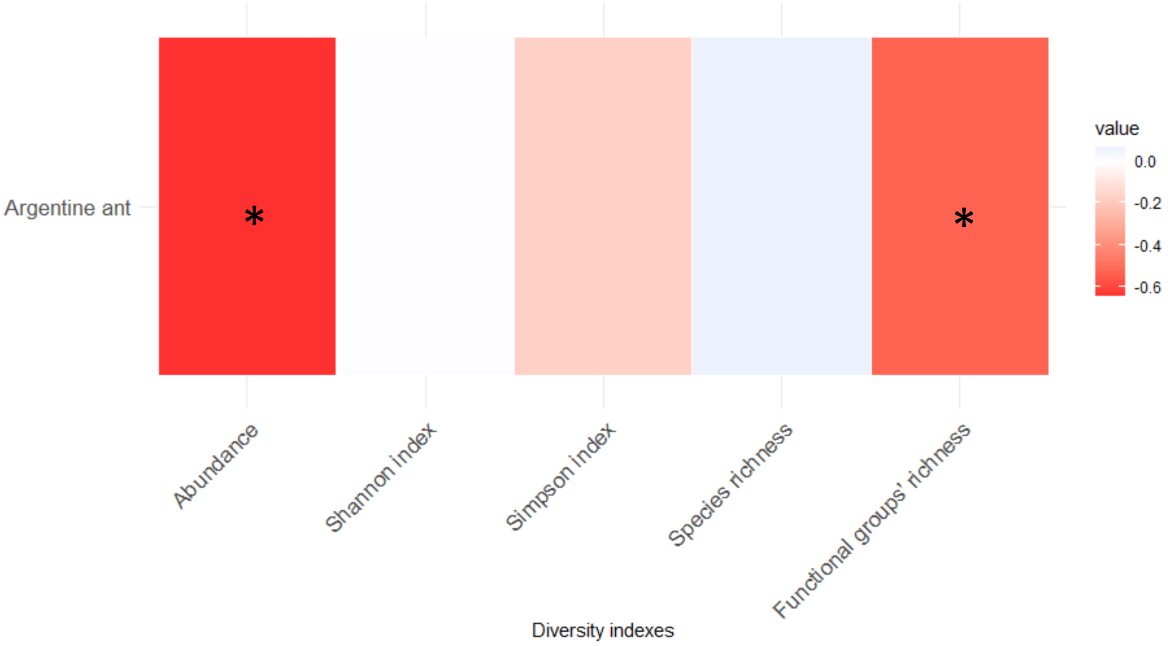

Figure 3.6 - Association of the Argentine ant presence and the diversity indexes of native ant communities using Spearman correlation tests. The * indicates significant values ($p < 0.05$).

Environmental data also shaped species' spatial distribution (Table 3.6). The PCA reduced the variables collected and the first four components were used. PCA1 was mainly explained positively by the plant-related variables (functional richness, and dissimilarity, Rao's quadratic entropy and plant's height cwm), PCA2 was positively explained by the plant richness and soil pH and negatively by organic matter and canopy cover, PCA3 was negatively explained by the proportion of sand in the soil and by plant functional evenness, and PCA4 was positively explained by plant richness, organic matter, and canopy cover. PCA2 and PCA4 both have a strong positive correlation with plant richness and negative correlations with organic matter and canopy cover (see Figures 6.12 to 6.17 in Supplementary material).

The models demonstrated the importance of environmental factors in driving ant species' distribution. All ten species modelled showed significant variations in occurrence due to environmental factors, except two (*Plagiolepis schmitzii* and *Solenopsis* sp.) as shown in Table 3.6.

Table 3.6 - GLMM results for the PCA components' effect on ants and the impact of the Argentine ant, *Linepithema humile*, on native ants. Significant values (P < 0.05) are highlighted.

	PCA1		PCA2		PCA3		PCA4		*Linepithema humile*		GLMM formula
	Estimate	P-value	Estimate	P-value	Estimate	P-value	Estimate	P-value	Estimate	P-value	
Linepithema humile	**1.24**	**< 0.01**	**-0.52**	**0.01**	**-0.62**	**< 0.01**	**0.63**	**0.02**	-	-	*PCA1* + *PCA2* + *PCA3* + *PCA4* + *Distance* + (1 | *Road*)
Tetramorium sp.	**0.23**	**0.04**	**-0.31**	**0.03**	**0.75**	**< 0.01**	-	-	**-0.97**	**0.01**	*PCA1* + *PCA2* + *PCA3* + *Distance* + *Line_hum* + (1 | *Road*)
Tapinoma erraticum	**-0.76**	**< 0.001**	0.29	0.08	**0.90**	**< 0.01**	-	-	-	-	*PCA1* + *PCA2* + *PCA3* + *Distance* + (1 | *Road*)
Temnothorax sp.	**0.32**	**0.047**	**-0.66**	**< 0.01**	**-0.86**	**< 0.01**	-	-	-	-	*PCA1* + *PCA2* + *PCA3* + *Distance* + (1 | *Road*)
Crematogaster scutellaris	**-0.76**	**< 0.01**	**-0.91**	**< 0.01**	-	-	-	-	-	-	*PCA1* + *PCA2* + *Distance* + (1 | *Road*)
Messor barbarus	-0.40	0.26	**1.21**	**< 0.01**	-	-	-	-	-	-	*PCA1* + *PCA2* + *Distance* + (1 | *Road*)
Plagiolepis schmitzii	-0.31	0.36	0.35	0.18	-	-	-	-	**-1.82**	**< 0.01**	*PCA1* + *PCA2* + *Distance* + *Line_hum* + (1 | *Road*)
Solenopsis sp.	-0.64	0.14	-0.48	0.11	-	-	-	-	**-3.30**	**< 0.01**	*PCA1* + *PCA2* + *Distance* + *Line_hum* + (1 | *Road*)
Aphaenogaster gibbosa	-0.001	1.00	**-0.61**	**0.01**	-	-	-	-	**-1.14**	**0.03**	*PCA1* + *PCA2* + *Distance* + *Line_hum* + (1 | *Road*)
Tapinoma nigerrimum	**-1.01**	**0.03**	**0.90**	**< 0.01**	-	-	-	-	**-2.83**	**< 0.01**	*PCA1* + *PCA2* + *Distance* + *Line_hum* + (1 | *Road*)

4. Discussion

The primary goal of this dissertation was to investigate the impact of road proximity on local ant communities. The hypothesis being tested was that ant communities located farther from roads would exhibit differences in composition compared to those near roads. The study experienced considerable challenges with several damaged traps by wild boars and the dominance of the invasive Argentine ant in some sites. These issues limited the depth of the results analysis. However, despite these shortcomings, the findings still provide valuable insights into certain patterns worth discussing.

4.1. Road distance effect on communities and species

The distance of the road did not seem to affect the ant community. The diversity indexes used to describe the ant community at different distances from the road showed that there are no significant differences between communities.

However, road distance did have an impact on some ant species and functional groups. This response to the road was seasonal, as some functional groups shifted their distribution as the seasons changed. For example, the relative abundance of the HCS/OH group was very low in Autumn, however in the Winter they showed a higher relative abundance far away from the road. This variation can be explained due to the limited availability of food in the Winter as well as the temperatures in this season being much lower. This trend changed in the Spring. This can be explained by the changes in the environmental conditions, with an increase of the foraging behaviour by these species. This proved their open-habitat specialization as the canopy cover near the road is much lower than far away from it (see table 6.2 in Supplementary material). In contrast, the CCS/SH were not as abundant as the former group, only appearing in the Spring. This goes against their expected abundance in colder seasons. This shift in the usual yearly fluctuation may suggest that a change in the temperature may impact ants, as a lot of species are highly heat-dependent for brood nursing (Brian, 1973; Penick and Tschinkel, 2008).

Thus, it was found no evidence that road distance is an important factor for the dissimilarities between ant communities, but this was probably masked by the high incidence of the invasive Argentine ant. In fact, the road N10 had a distinct ant community, most probably due to the low abundance and incidence of the invasive species. The ant communities showed distinct biodiversity patterns across roads. Road N10 was the richest in species and showed no dominance of particular species. On the contrary, road N119 had the highest Argentine ant abundance, being the road where the impact of the invasive ant was more notable. Significant differences were found between N10 and the other two roads for the Shannon and Simpson indexes, indicating a shift in the composition of ant communities amongst roads. This effect could be explained by the presence of cattle in N118 and N119. Grazing can affect soil composition as well as vegetation structure and composition (Listopad et al., 2018). Thereby, ant communities can suffer significant variations in structure and composition with the homogenization of their environment as invasive species may adapt better to these conditions. This goes in line with the work of Frasconi Wendt et al. (2021a), who studied the changes in ant communities in a post-grazing succession in the same sampling area and found an increase in species richness in sites with greater year grazing exclusion, where vegetation structure was more complex. Opposite results were found by Eldridge et al. (2020) in Australia since they showed that increasing grazing intensity led to greater ant species richness due to changes in litter depth and grass density.

4.2. Drivers of ant community structure and composition

The vegetation had an important role in shaping the ant community. Several environmental variables have a pivotal role in shaping the ant community structure and composition. These factors were not associated with road distance as different species with similar road responses demonstrated different responses to the same variables. The invasive species, *Linepithema humile*, prefer environments with a taller and more diverse vegetation, a higher canopy cover and a soil composed mainly by finer particles. *Tapinoma erraticum* showed opposite trends for all environmental factors. *Crematogaster scutellaris* and *Temnothorax* sp. evidence contrary trends regarding vegetation height structure. This goes to show a micro-scaled species response instead of a macro-scaled community response. Ants may vary their distribution according to micro-scale variations in environmental factors, as its demonstrated by Frasconi Wendt et al. (2021c) where along a micro-scale gradient ant functional structure and diversity changed.

The Argentine ant is a highly successful invasive species that has spread throughout much of the world. Originally native to South America, this ant species has established large supercolonies in many parts of the world, including Europe, Australia, and North America (Wetterer et al., 2009). It has been present in Portugal since 1890 (Dias, 1955) and is now widely distributed throughout the country (Espadaler and Gómez, 2003). It is particularly abundant in urban and suburban areas but has also been reported in agricultural and natural environments (Holway, 1998; Vega and Rust, 2000; Touyama et al., 2003). The success of the Argentine ant can be attributed to several factors, including its ability to form massive, interconnected colonies, its adaptability to a wide range of environments (Van Wilgenburg et al., 2010; Moffet, 2012), and its aggressive behaviour towards other ant species (Paiva, 1998; Holway, 1999; Human and Gordon, 1999; Carpintero et al., 2005). The Argentine ant has had significant ecological and economic impacts (Barber, 1921; Ward et al., 2010) in areas where it has become established, including displacement of native ant species, disruption of ecosystem processes, and damage to crops.

As described in De Kock and Giliomee (1989) the Argentine ant is frequently found near roads or other disturbed environments. However, in this study, the differences in this species' abundance in the different distances were not significant, showing that the Argentine ant has already expanded (especially on road N119), exploring, and dominating most resources far away from the road. This expansion trend is expected due to the species' social organization (polygyny and ability to form supercolonies) (Ingram, 2002; Van Wilgenburg et al., 2010).

If the Argentine ant has a similar incidence near and far from roads, then in areas with fewer conditions for this species to thrive, road verges may provide effective dispersal corridors. Verges can retain high humidity (northern slopes) which may be used by this species to disperse. This is suggested in the work of Heller and Gordon (2006), where it was proven that the spatial distribution of colonies of the Argentine ant fluctuate seasonally and that these ants may prefer humid environments (verges) rather than those close to food sources. Moreover, as some native species seem to avoid road verges, the resistance driven by local native communities may be lower along verges.

The impact of the Argentine ant on native ant fauna (Holway, 1999) was confirmed in this study with this species being negatively correlated with almost all species captured. Native species seem to have their distribution restricted by the invasive ant, as the presence of the Argentine ant negatively impacted ant richness and abundance. An impacted native ant community could imply a deterioration in ecosystem services such as seed dispersal (Gómez and Oliveras, 2003; Fransconi Wendt et al., 2022), one of the most important plant-animal mutualisms (Handel and Beattie. 1990). This ecosystem service is also often impacted by habitat fragmentation (Bona et al., 2023). Therefore, seed dispersal promoted by ant communities inhabiting road vicinities can be threatened by the presence of invasive species (like the Argentine ant) and road disturbance effects.

Regarding the ant community, the Argentine ant seems to impact the overall ant abundance and functional groups' richness, as some functional groups were outcompeted due to the aggressiveness of the invasive ant (Figure 3.2). Predator specialists was the most affected group by the presence of this invasive ant. Ants from that functional group can be losing ecological space to the Argentine ant in this type of Mediterranean ecosystem as reported in other studies (Holway, 1999; Gómez and Oliveras, 2003). Future research should focus on the impact of this invasive species on the different functional groups and its consequences for ecological processes.

4.3. Conservation Implications

In conclusion, road verges play a crucial role in local communities as it serves as an ecological corridor for several species, including non-natives due to a higher perturbation at these sites. Nevertheless, this phenomenon tends to be seasonal in some ant species. The presence of the Argentine ant presents a serious threat to the native ant fauna as it impacts the distribution of species directly (aggressive behaviour) and indirectly (competition for the same resources) as well as the ecosystem functioning as it disrupts some ecosystem services provided by native ants, such as seed dispersal. Interestingly, N10 presented lower numbers of the invasive ant as its surrounding environment is more natural and less impacted by cattle grazing. It is therefore suggested a sustainable management of cattle grazing with implementation of cattle-free parcels. These parcels can serve as a source for populations of native invertebrate species affected by the homogenization of the vegetation, as grazing can directly impact the distribution of native species and promotes the expansion of invasive ones. The importance of this type of land management increases with the presence of roads as it acts as an additional disturbance to the ecosystem. The species inhabiting road verges may take advantage with these types of habitats but may be losing environmental fitness (i.e. less fertility or longevity) or accumulating toxic substances compared to populations of the same species inhabiting far away from roads. Therefore, it is suggested a more in depth study of the biology of the species living in road vicinities. It is also necessary a stricter control of the invasive Argentine ant in places where its presence is more problematic for the normal function of the ecosystem. This can be done by controlling and managing some environmental conditions that can favour the establishment of this species, like the soil disturbance caused by cattle grazing mentioned before. The constant challenge in improving ant communities invaded by the Argentine ant can be eased by a better understanding of the ecology of species that may be more resistant to this invasive species. This study is focused on ants as a bioindicator group but is likely that roads have similar effects on other invertebrate groups. This work provides a framework for future studies to develop research on how roads impact invertebrate communities while disentangling the impact of invasive species on the road effect.

5. References

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6. Supplementary material

Table 6.1 – Traps' location by road, distance and replicate.

Site	Replicate	Coordinates	
		Latitude	**Longitude**
N10			
0 m	1	38°50'44.02"N	8°45'31.47"W
	2	38°50'52.25"N	8°46'12.03"W
	3	38°51'50.35"N	8°47'5.72"W
	4	38°53'8.51"N	8°49'20.28"W
	5	38°53'4.87"N	8°49'14.78"W
	6	38°50'43.73"N	8°45'40.34"W
50 m	1	38°50'42.26"N	8°45'32.07"W
	2	38°50'51.03"N	8°46'13.22"W
	3	38°51'49.91"N	8°47'7.89"W
	4	38°53'7.66"N	8°49'21.78"W
	5	38°53'3.60"N	8°49'15.66"W
	6	38°50'41.98"N	8°45'40.65"W
100 m	1	38°50'41.10"N	8°45'31.30"W
	2	38°50'50.03"N	8°46'14.39"W
	3	38°51'49.37"N	8°47'8.97"W
	4	38°53'6.30"N	8°49'22.57"W
	5	38°53'2.53"N	8°49'17.34"W
	6	38°50'40.67"N	8°45'41.48"W
N118			
0 m	1	38°50'26.83"N	8°52'51.05"W
	2	38°50'52.57"N	8°52'51.12"W
	3	38°51'0.39"N	8°52'51.07"W
	4	38°50'16.23"N	8°52'51.10"W
	5	38°50'21.51"N	8°52'51.09"W
	6	38°50'31.01"N	8°52'50.82"W
50 m	1	38°50'26.83"N	8°52'47.94"W
	2	38°50'52.23"N	8°52'48.88"W
	3	38°51'0.04"N	8°52'48.66"W
	4	38°50'16.41"N	8°52'48.71"W
	5	38°50'21.61"N	8°52'48.50"W
	6	38°50'31.06"N	8°52'48.12"W
100 m	1	38°50'26.52"N	8°52'45.90"W
	2	38°50'51.91"N	8°52'47.18"W
	3	38°51'0.24"N	8°52'46.68"W
	4	38°50'15.91"N	8°52'46.96"W

	5	38°50'21.92"N	8°52'46.76"W
	6	38°50'30.44"N	8°52'46.36"W
N119			
0 m	1	38°47'26.40"N	8°50'12.14"W
	2	38°47'47.34"N	8°49'27.32"W
	3	38°48'34.06"N	8°47'47.99"W
	4	38°48'30.72"N	8°47'55.04"W
	5	38°47'54.33"N	8°49'13.89"W
	6	38°47'23.92"N	8°50'18.04"W
50 m	1	38°47'27.78"N	8°50'13.16"W
	2	38°47'49.63"N	8°49'27.70"W
	3	38°48'35.74"N	8°47'48.66"W
	4	38°48'32.10"N	8°47'56.33"W
	5	38°47'55.85"N	8°49'14.41"W
	6	38°47'25.21"N	8°50'18.98"W
100 m	1	38°47'29.40"N	8°50'14.21"W
	2	38°47'51.06"N	8°49'29.10"W
	3	38°48'37.49"N	8°47'48.53"W
	4	38°48'33.69"N	8°47'57.52"W
	5	38°47'56.74"N	8°49'16.58"W
	6	38°47'26.81"N	8°50'20.61"W

Figure 6.1 – Worker of *Proceratium melinum* captured. A: Lateral view, B: Head view, C: Dorsal view. Photos by Roberto Keller.

Table 6.2 - Environmental variables collected and their average per road and distance. Sand proportion, Organic matter and Canopy cover values are presented in %

	Plant richness	Functional richness	Functional evenness	Functional dispersion	Rao's quadratic entropy	Sand proportion	Organic matter	Soil pH	Canopy cover
Road N10	12.44	2.10	0.53	0.41	0.31	80.63	0.75	5.86	23.23
0	15.83	2.01	0.51	0.38	0.27	81.74	0.70	6.59	13.23
50	11.67	2.11	0.51	0.43	0.33	80.62	0.83	5.50	25.01
100	9.83	2.17	0.56	0.42	0.34	79.52	0.72	5.47	31.44
Road N118	12.11	2.57	0.58	0.54	0.55	82.77	1.29	5.85	21.71
0	10.67	3.41	0.58	0.78	1.03	84.90	1.64	6.25	12.79
50	13.83	2.25	0.61	0.43	0.33	82.30	1.20	5.74	21.13
100	11.83	2.05	0.54	0.40	0.29	81.11	1.01	5.56	31.20
Road N119	12.94	2.36	0.56	0.46	0.45	86.03	1.18	5.93	32.71
0	16.67	2.27	0.52	0.41	0.32	86.49	0.91	6.27	17.28
50	12.00	2.98	0.56	0.54	0.74	84.03	1.38	5.78	36.38
100	10.17	1.84	0.60	0.42	0.28	87.56	1.26	5.73	43.75

Table 6.3 - Traps that were destroyed per distance and road in each season

Site	Traps destroyed	
	Autumn	**Spring**
Road N10		
0 m	0	0
50 m	0	0
100 m	46,7%	0
Road N118		
0 m	0	33,3%
50 m	6,7%	33,3%
100 m	26,7%	0
Road N119		
0 m	0	20%
50 m	0	3,3%
100 m	6,7%	20%
Total	**13**	**33**

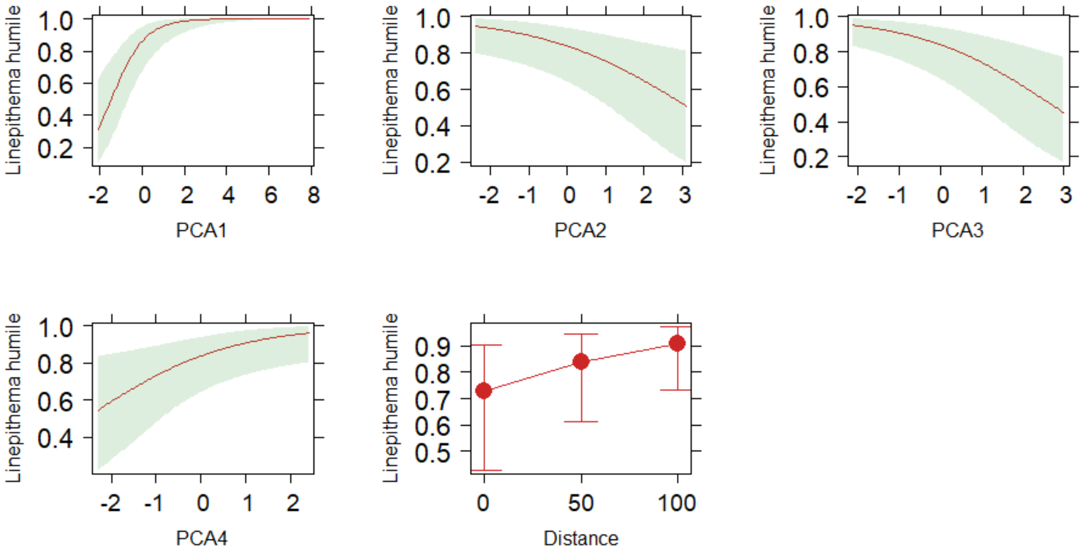

Figure 6.2 - GLMM output for the variables used for *Linepithema humile*.

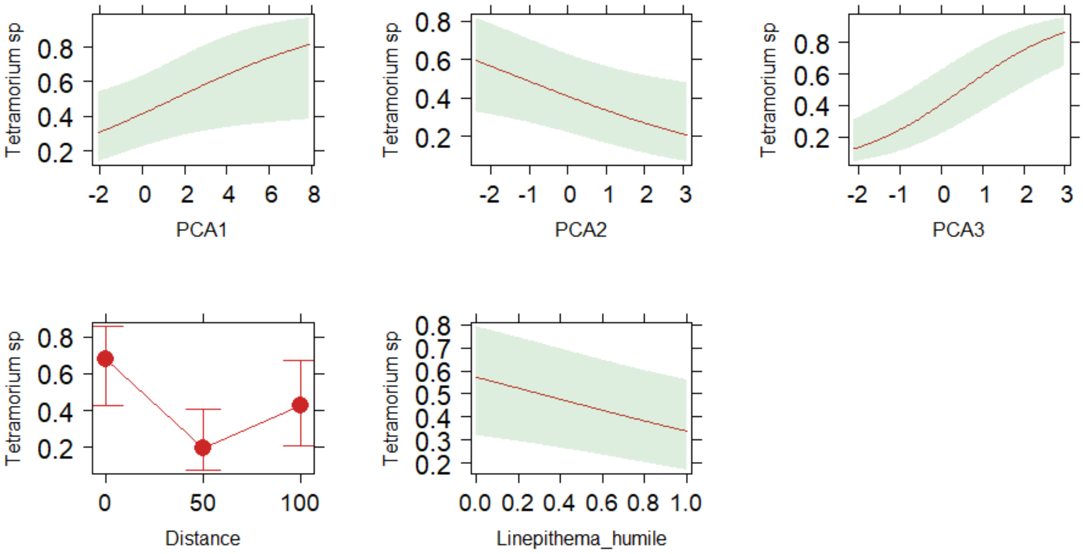

Figure 6.3 - GLMM output for the variables used for *Tetramorium* sp.

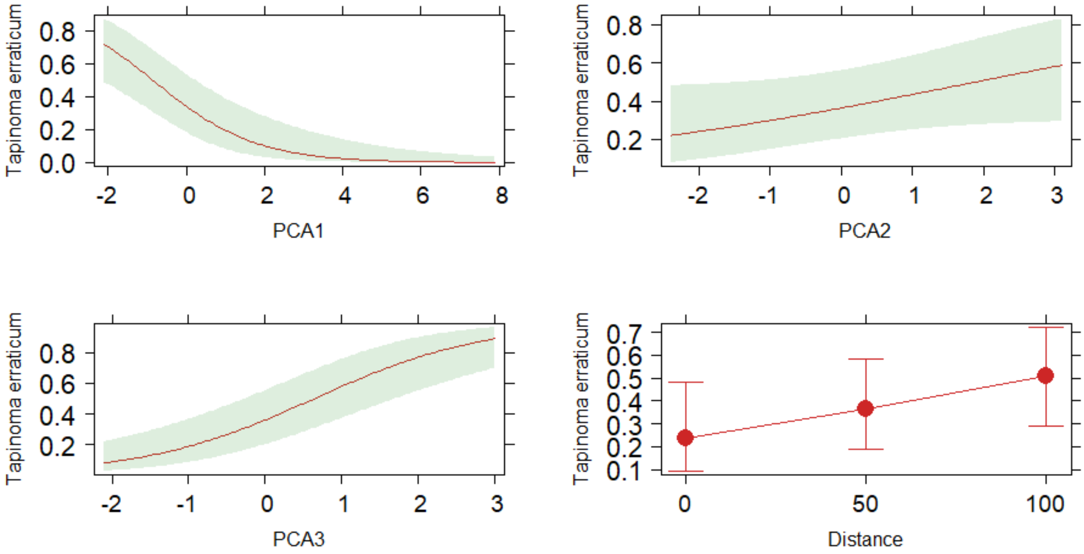

Figure 6.4 - GLMM output for the variables used for *Tapinoma erraticum*.

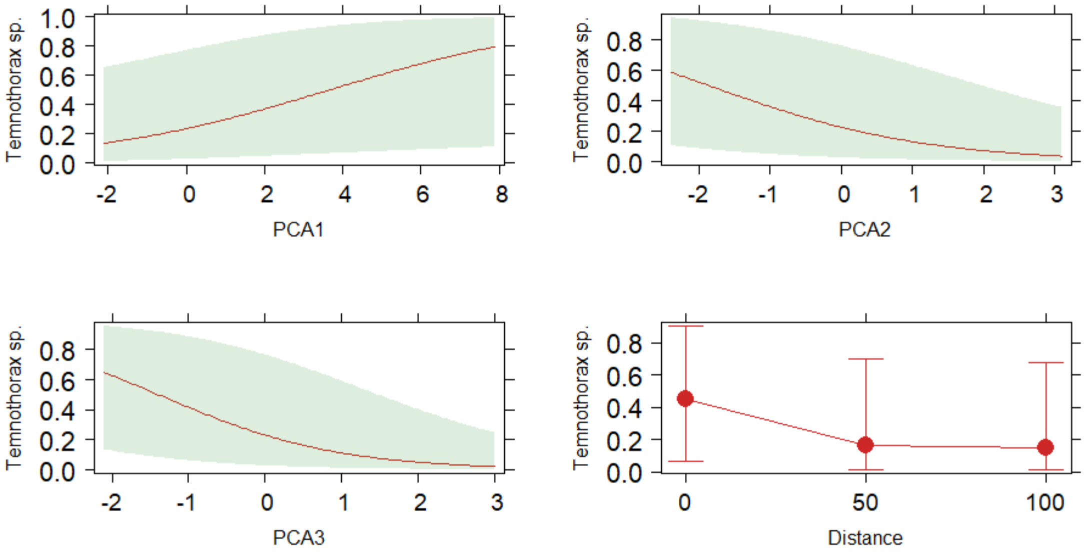

Figure 6.5 - GLMM output for the variables used for *Temnothorax* sp.

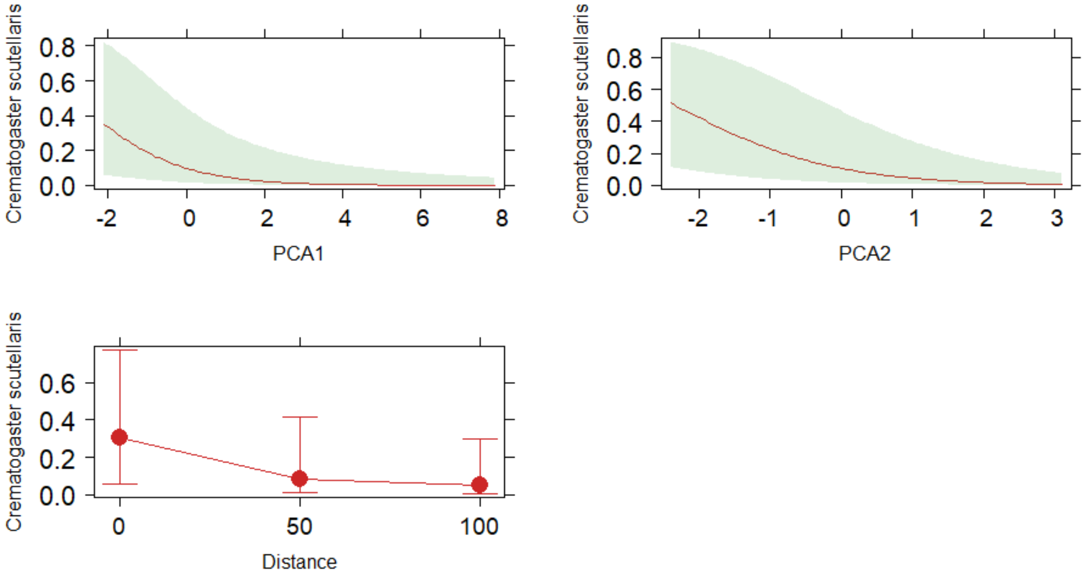

Figure 6.6 - GLMM output for the variables used for *Crematogaster scutellaris*.

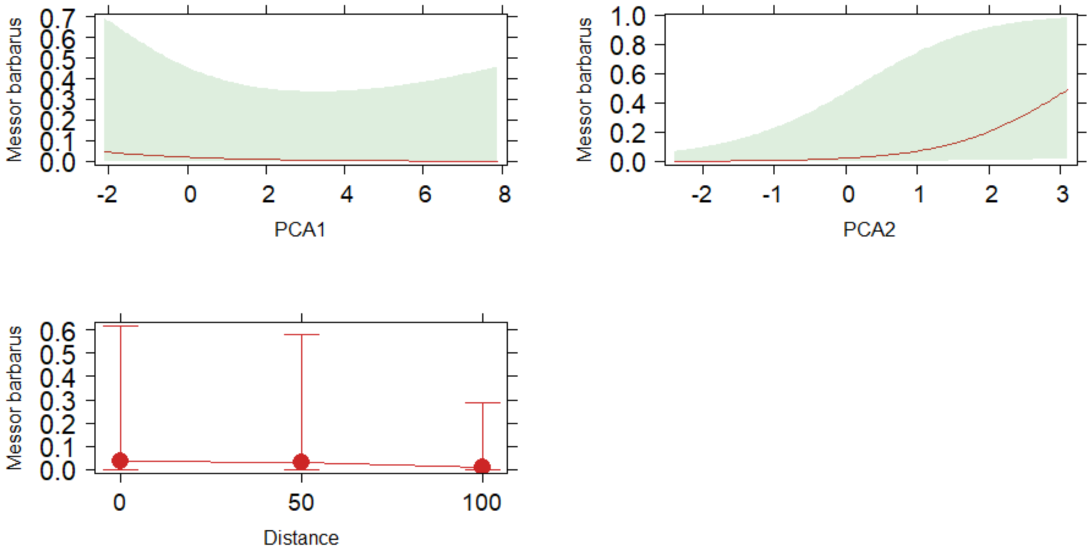

Figure 6.7 - GLMM output for the variables used for *Messor barbarus*.

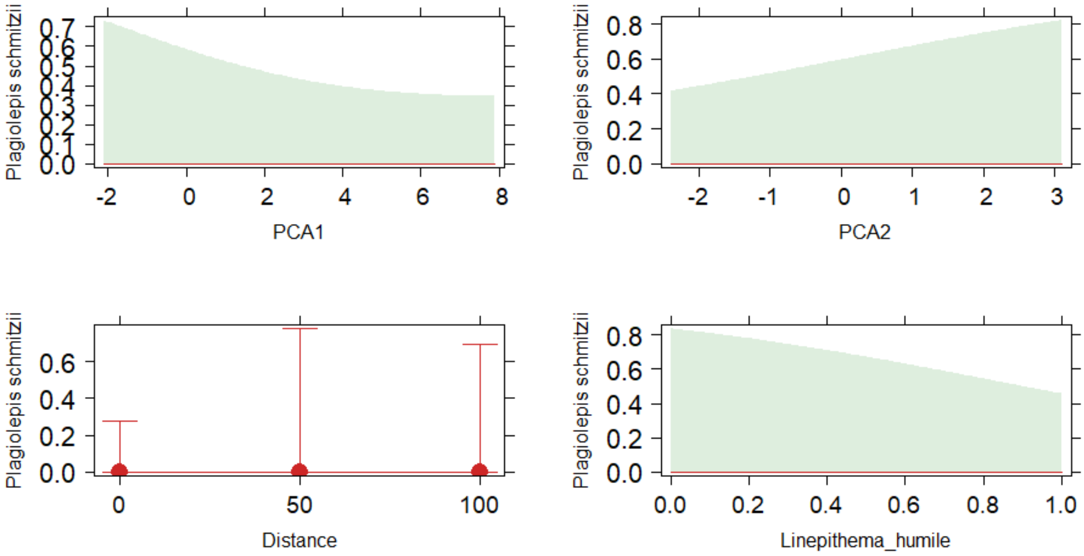

Figure 6.8 - GLMM output for the variables used for *Plagiolepis schmitzii.*

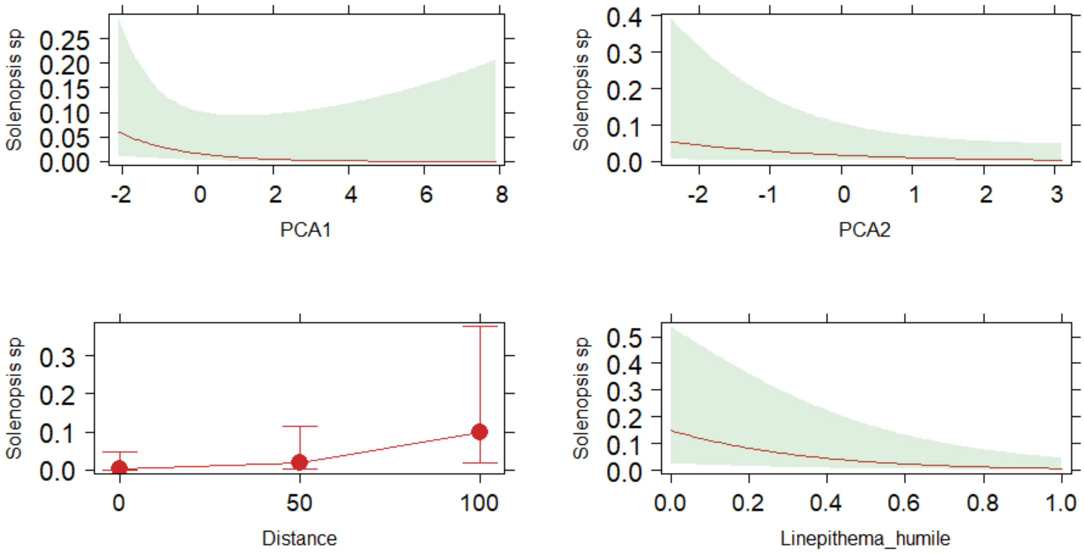

Figure 6.9 - GLMM output for the variables used for *Solenopsis* sp.

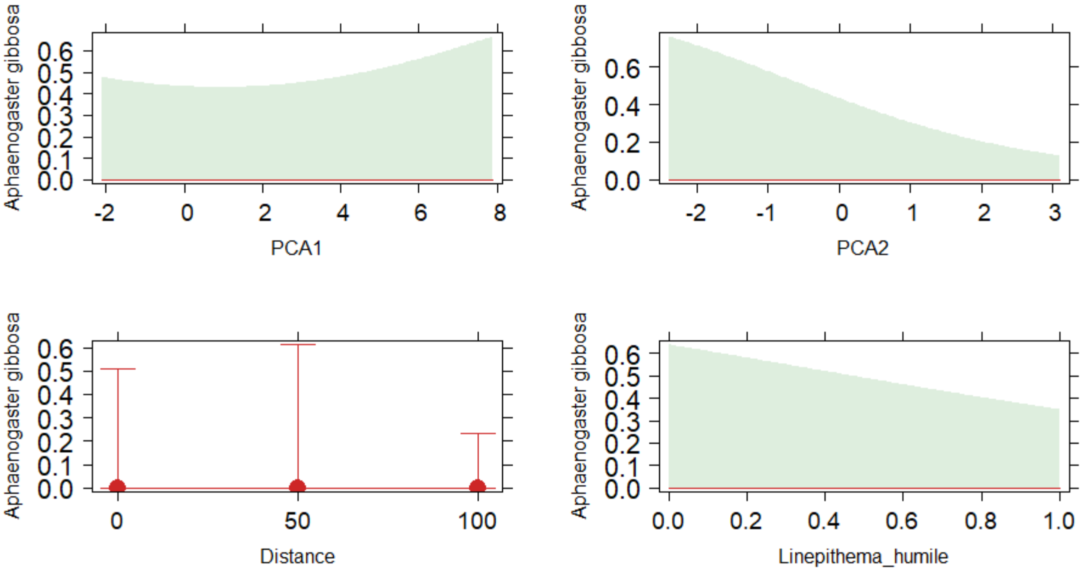

Figure 6.10 - GLMM output for the variables used for *Aphaenogaster gibbosa*.

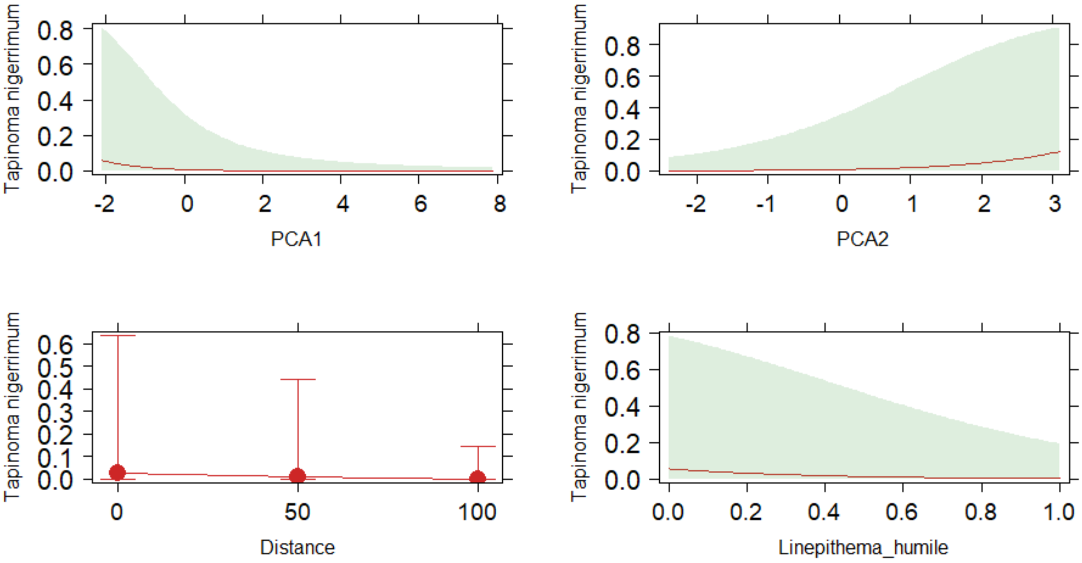

Figure 6.11 - GLMM output for the variables used for *Tapinoma nigerrimum*.

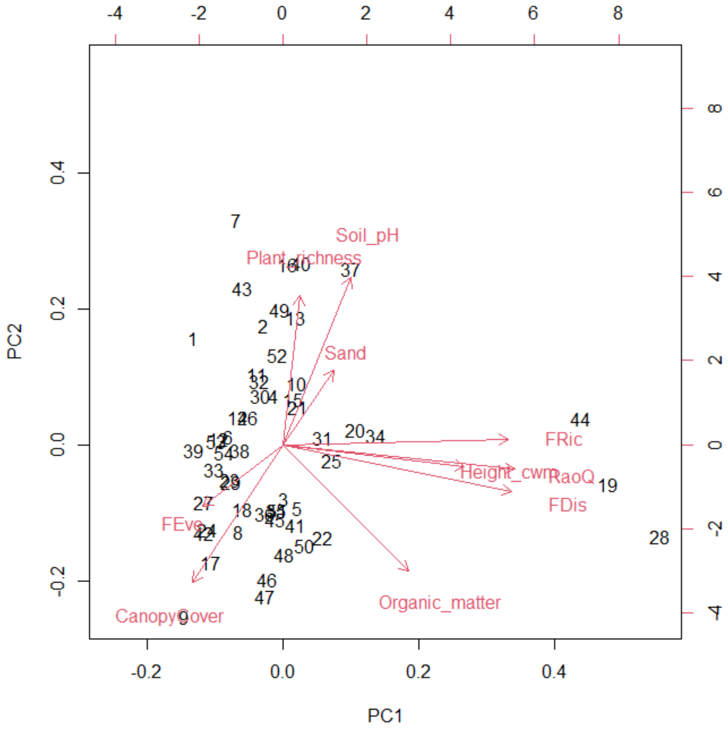

Figure 6.12 - Visualization of the different variables' contribution to PCA components 1 and 2.

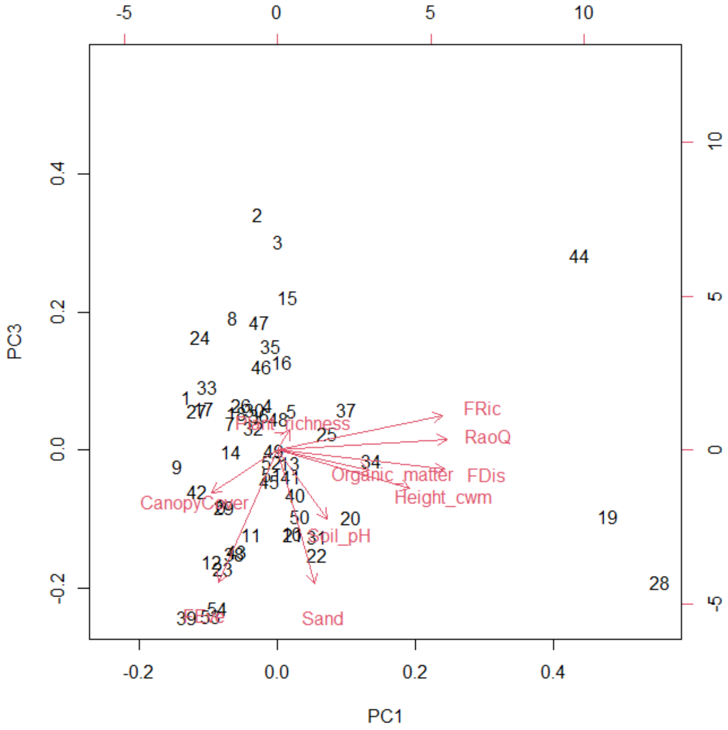

Figure 6.13 - Visualization of the different variables' contribution to PCA components 1 and 3.

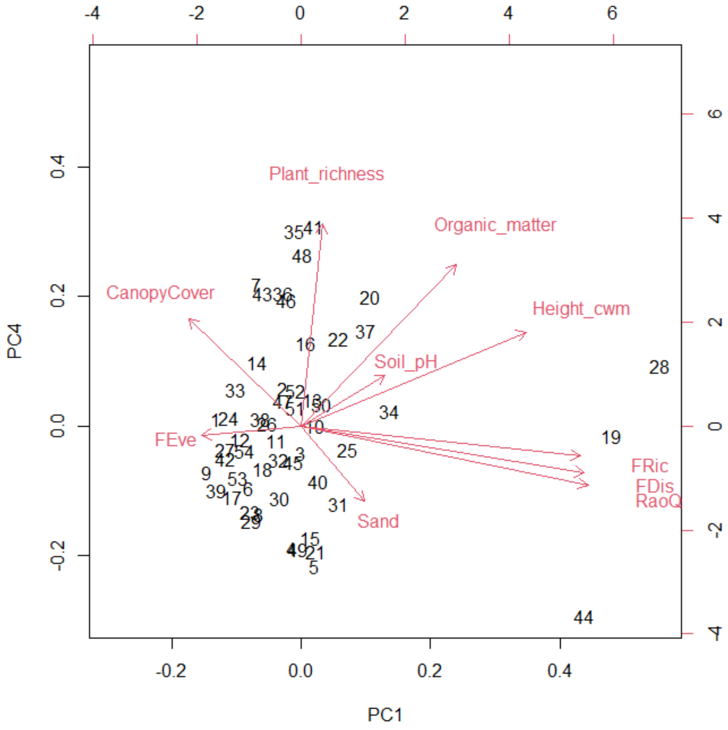

Figure 6.14 - Visualization of the different variables' contribution to PCA components 1 and 4.

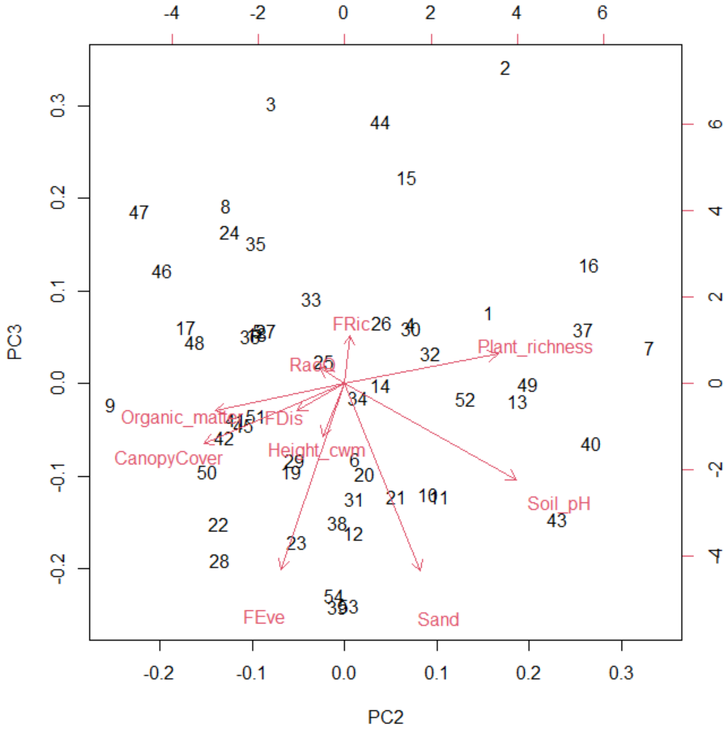

Figure 6.15 - Visualization of the different variables' contribution to PCA components 2 and 3.

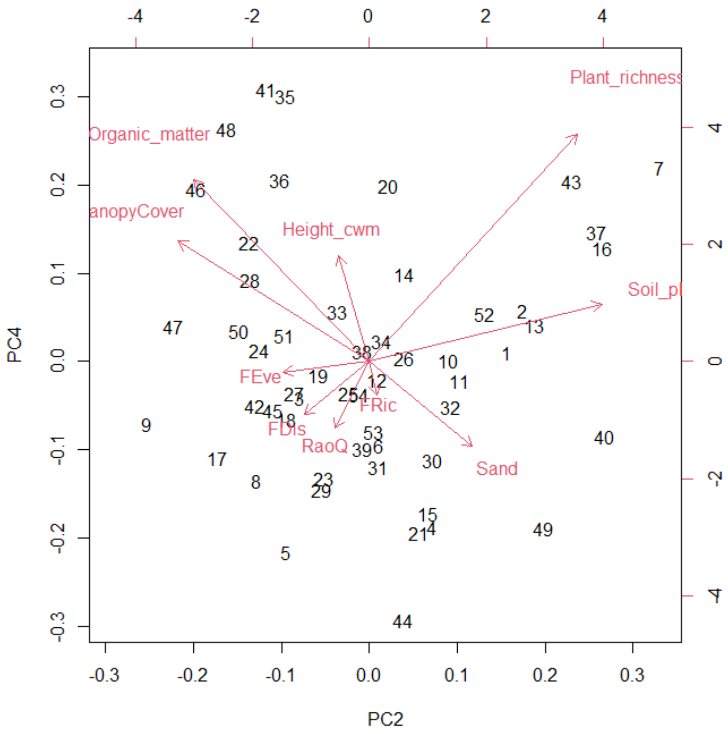

Figure 6.16 - Visualization of the different variables' contribution to PCA components 2 and 4.

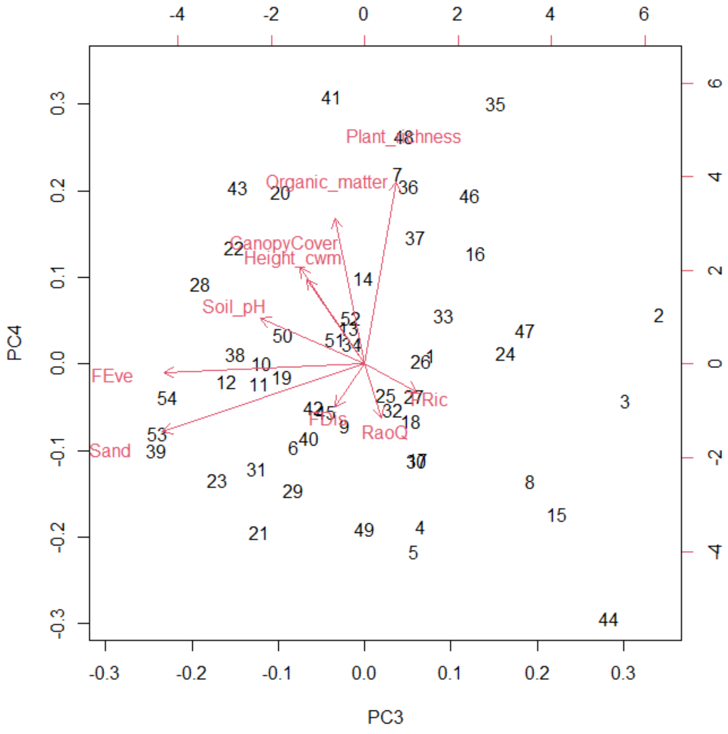

Figure 6.17 - Visualization of the different variables' contribution to PCA components 3 and 4.