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Assessing arthropod species/groups as bioindicators of ecosystem health in the native forests of Terceira Island

Màster en Canvi Ambiental i Transició Socioecològica

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Date of submission of the memoir through the TFM platform: 05/09/2024

SUMMARY

Abstract

Global changes are profoundly affecting biodiversity worldwide, with human activities like resource exploitation, pollution, and habitat fragmentation playing significant roles. These impacts are especially dramatic on islands due to their unique ecological characteristics and the higher vulnerability of insular species to extinction. Understanding the status of vulnerable habitats is crucial for all species' well-being and effective conservation strategies. This study aims to evaluate the Index of Biotic Integrity (IBI) across various sites on Terceira Island (Azores) and reliably identify key arthropod species that indicate ecological conditions. We used data from two comprehensive projects—the SLAM (entitled "SLAM project - Long Term Ecological Study of the Impacts of Climate Change in the Natural Forests of the Azores") project and the BALA (Biodiversity of Arthropods from Laurisilva of the Azores) project—to analyse arthropod biodiversity and its implications for forest conservation. Different sampling methods, including SLAM traps and canopy beating, provided a multifaceted understanding of the arthropod communities.

Using a generalised linear mixed model (GLMM) for both databases, we identified specific arthropod species, such as *Pinalitus oromii* and *Ommatoiulus moreleti*, as key bioindicators of forest health. *Pinalitus oromii*, an endemic species, showed a positive correlation with higher IBI values, suggesting its potential as a reliable indicator of pristine forest conditions. In contrast, *Ommatoiulus moreleti*, an invasive species, exhibited a negative relationship with IBI values. These findings highlight the importance of integrating multiple ecological indicators and biogeographic categorizations in monitoring forest health, providing valuable insights for conservation strategies in the Azorean native forests.

The study further emphasises the need to select and analyse various environmental variables such as altitude, distance to roads, and forest structure, which significantly influence habitat quality and biodiversity. By employing a comprehensive dataset from long-term monitoring projects, this research offers a robust analysis of arthropod community dynamics over time. The application of the IBI tailored for the Azorean forest ecosystem incorporates diverse taxonomic and ecological parameters, facilitating a multimetric evaluation of biological integrity. Comparative analyses using both the IBI-SLAM and IBI-Canopy indices across different forest strata ensure a thorough assessment of forest biodiversity and ecosystem health.

Our study underscores the importance of selecting appropriate bioindicator species for effective biodiversity monitoring and conservation efforts. It suggests that certain arthropod species can be used as indicators of native forest conditions with high ease of sampling and identification. These results have significant implications for conservation strategies on Terceira Island, laying the groundwork for future research and the development of effective management plans to preserve the Azores' unique biodiversity.

1. Introduction

Global changes are exerting significant impacts on biodiversity at a worldwide scale (Habibullah et al., 2022; O'Connor et al., 2020; Shivanna, 2022). Human activities have been empirically shown to significantly contribute to biodiversity loss (Brown & Pearce, 2023; Jaureguiberry et al., 2022). Fragmentation, degradation, the introduction of invasive species, resource exploitation, pollution, and climate change are key factors driving this decline (Christian, 2023; Hirt et al., 2021; Jaureguiberry et al., 2022; Kumar Rai & Singh, 2020). Of these, land/sea use change has been identified as the primary direct driver of recent biodiversity loss globally. Direct exploitation of natural resources ranks second, followed by pollution. While climate change and invasive alien species are significant factors, their impact has been less pronounced compared to the top two drivers (Jaureguiberry et al., 2022). Although climate change is relevant, a recent analysis of vertebrate extinctions and threat data from the IUCN Red List reveals that habitat loss and overexploitation pose greater risks to biodiversity than climate change (Caro et al., 2022).

Biodiversity loss on islands is even more dramatic due to their unique ecological characteristics and the higher vulnerability of insular species to extinction (Kier et al., 2009; Whittaker & Fernandez-Palacios, 2007). This heightened vulnerability is largely attributed to the small population sizes, limited geographic ranges, and specialised niches of island species (Pimm et al., 2014). The isolation of life on islands has led to distinct evolutionary pathways, resulting in numerous unique lineages and communities that are exclusive to these regions (Losos & Ricklefs, 2009). Island plants and vertebrates exhibit an endemic richness that may exceed that of mainland species by a factor of 9.5 (Spatz et al., 2022), also with significant levels of endemism across various taxonomic groups, thereby making a substantial contribution to global biodiversity (Condamine et al., 2017; Kier et al., 2009; Whittaker & Fernandez-Palacios, 2007; Wilmé et al., 2006). Although comprising only 6.7% of the Earth's land surface, these regions host approximately 20% of the planet's biodiversity. Regrettably, they also account for approximately 50% of threatened species and have witnessed 75% of known extinctions since the era of European expansion across the globe (Fernández-Palacios et al., 2021). Past extinctions were likely triggered by a combination of factors including invasive species, the naïveté of insular species to novel threats, and the restricted geographic ranges of certain populations (Caujapé-Castells et al., 2010; Cox & Lima, 2006; Frankham, 1998; Wilmé et al., 2006; Wulff et al., 2013). Currently, biodiversity on islands is experiencing a substantial decline due to multiple drivers of biodiversity erosion (Simberloff, 2000) which include habitat loss, degradation, and fragmentation, as well as climate change and the introduction and proliferation of invasive species (Borges et al., 2019; Fernández-Palacios et al., 2021).

A significant component of unique island biota consists of invertebrates, which were able to easily colonise islands through long-distance dispersal mechanisms, as discussed by Carlquist (1966). Living arthropods comprise more than one million species and represent the majority of Earth's animal richness (Hedges & Kumar, 2009). The estimated total number of arthropod species on Earth is approximately 6.8 million (range 5.9-7.8 million), with a significant percentage of these species (around 85%) still awaiting discovery and description (Stork et al., 2015). Notwithstanding, arthropods are experiencing dramatic population declines and species extinctions worldwide (Cardoso et al., 2020; Harvey et al., 2020; Sánchez-Bayo & Wyckhuys, 2019). The primary causes of arthropod decline, as previously discussed, include habitat destruction, intensive agriculture, pesticide use, pollution, climate change, and the introduction of invasive species (Dar et al., 2021). Insects represent approximately 80% of extant species and exert a multifaceted impact on humanity. They provide crucial ecological services vital to agriculture, including pollination, and are also significant vectors of pathogenic microorganisms affecting animal and human health. Additionally, insects play a pivotal role in biogeochemical cycles and contribute to the regulation of vertebrate populations (Jactel et al., 2020), so the loss in insect diversity is a risk to both ecosystem sustainability and human health and well-being (Sandifer et al., 2015). Despite their ecological significance, insect biodiversity and population dynamics often receive limited attention from ordinary people, policymakers, and local and national authorities (Cardoso et al., 2020). Urgent calls have been made for immediate action to address these declines and to establish standardised, long-term monitoring programs (Hallmann et al., 2019; Hallmann et al., 2017, 2021; Seibold et al., 2021; Wagner, 2020) considering that most insect population studies and reports have a pronounced geographical focus on Europe and North America. Consequently, there is a significant gap in our understanding of insect populations in other regions of the world, and the extent to which current findings are globally applicable remains uncertain (Borges, Rigal, et al., 2020).

Island populations, communities, and ecosystems are distinct, self-contained entities with clear geographical boundaries that encompass essential ecological processes (Vitousek et al., 2013). Additionally, islands have undergone profound habitat degradation because of human colonisation (Borges et al., 2019; Borges, Santos, et al., 2020; Triantis et al., 2010). The islands of the Azores archipelago have native island habitats that underwent significant modification following Portuguese settlement in the 15th century, with the replacement of pristine forests by pasturelands, agricultural areas, exotic tree plantations (e.g. *Cryptomeria japonica* and *Eucalyptus spp.*), and urban development (Borges, Lamelas-López, et al., 2022). These extensive land-use changes occurred gradually across elevational gradients, resulting in the extinction of large-bodied beetle species (Terzopoulou et al., 2015) and an ongoing process of extinction debt for many others (Triantis et al., 2010). Presently, the original forests cover only about 5% of the islands' total surface area and are restricted to the most inaccessible regions (Gaspar et al., 2008.; Norder et al., 2020; Rego et al., 2015; Triantis et al., 2010) with these remnants designated as protected areas for which conservation plans are needed soon (Gaspar et al., 2010).

In scientific research, biodiversity indicators serve as quantifiable environmental factors that facilitate the assessment of biodiversity responses to environmental changes, enabling the establishment of effective conservation and management strategies (Otomo et al., 2023). Given the inherent complexity of biodiversity, which is challenging to comprehensively measure and quantify in even a small area, it is essential to identify suitable indicators (Duelli & Obrist, 2003). The use of surrogates, typically a subset of species whose diversity or distribution can be indicative of the overall biodiversity (e.g., Araújo et al., 2004; Balmford & Long, 1995), is a popular shortcut for assessing and monitoring biodiversity in various areas. If these surrogates demonstrate reliable predictive properties, they can provide valuable tools for conservation planning and other purposes, particularly in contexts where financial and time resources are limited (Gaspar et al., 2010). Surrogates can be selected based on the specific characteristics of the community being predicted, whether it pertains to diversity (diversity surrogate), co-occurrence (biotic indicator), habitat quality (environmental indicator) or taxa (higher taxa surrogate) (Caro et al., 1999; Gaston & Williams, 1993; Heino, 2006). Recently, in the Azores archipelago, an extensive standardised sampling protocol has been implemented across most forest fragments resulting in the largest standardised database of arthropods available for the Macaronesia region (encompassing the Azores, Madeira, Savage, Canaries, and Cape Verde archipelagos in the North Atlantic Ocean) (Gaspar et al., 2010). All the species recorded encompass distinct trophic groups, exhibit different colonisation histories, possess dissimilar dispersal abilities, and demonstrate varying abundances and distributional ranges (see also Gaspar et al., 2008.). Previous studies leveraging these data have evaluated the effectiveness of arthropods as higher taxa surrogates (Borges et al., 2005) and environmental indicators (Cardoso et al., 2007).

Long-term studies are essential for understanding the drivers of biodiversity erosion, including land use change, habitat degradation, climate change, invasive species, and pollution. The Azores (Portugal) is recognized as a Mediterranean biodiversity hotspot and is subject to various monitoring projects, including those conducted by the Azorean Biodiversity Group focusing on both native and exotic forest arthropods. The SLAM project (entitled "SLAM project

- Long Term Ecological Study of the Impacts of Climate Change in the natural forests of the Azores") commenced in 2012 and is currently monitoring arthropod populations using divers "Sea, Land, and Air Malaise" (abbreviated SLAM) traps (Figure 1), intending to comprehend the impact of biodiversity erosion drivers on the native forests of the Azores, Portugal (Borges, Lamelas-Lopez, & Schülke, 2022; Borges, Lamelas-Lopez, Stüben, et al., 2022; Borges, Lamelas-López, et al., 2022; Lamelas-Lopez et al., 2023; Lhoumeau, Cardoso, Boieiro, et al., 2022; Lhoumeau, Cardoso, Costa, et al., 2022). Complementing this initiative, the Biodiversity of Arthropods of the Laurisilva of the Azores (BALA) project, initiated in 1997, has also significantly contributed to the understanding of arthropod biodiversity in the Azores Islands. The BALA project employs standardised sampling methods across various islands to gather comprehensive data on arthropod populations, thereby addressing the scarcity of long-term arthropod datasets with the same purpose as the SLAM project (Pozsgai et al., 2024).

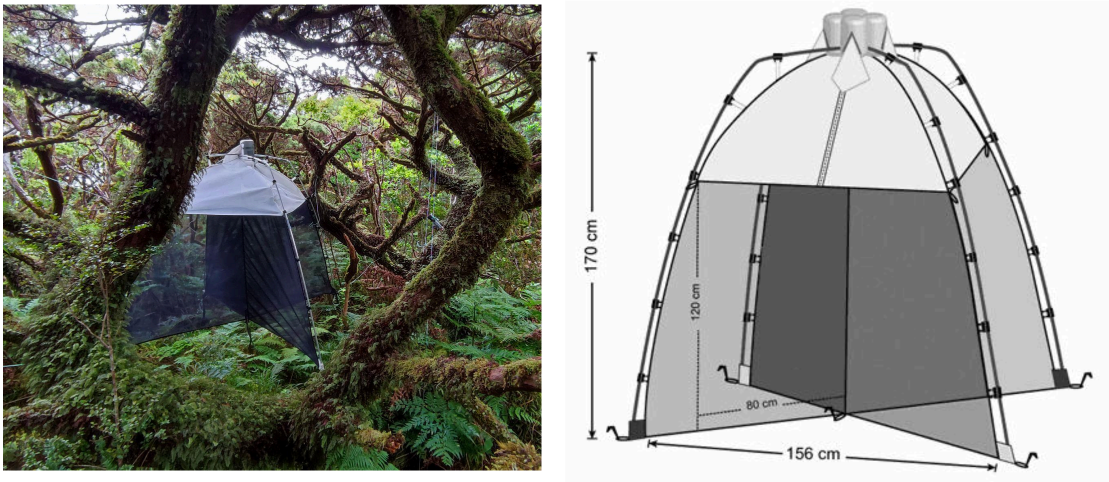

Figure 1. On the left a SLAM (Sea, Land, and Air Malaise) trap is positioned at ground level within a section of native forest. The ropes employed to support the structure can serve as pathways for flightless arthropods, resembling extensions of tree branches, aiding in their navigation within the intricate forest terrain. Additionally, ground-dwelling arthropods have the capability to ascend the SLAM structure to access the sample bottle. On the right, a diagram of the structure of the SLAM trap with the dimensions. Image from Lhoumeau & Borges (2023).

Understanding the status of surrounding habitats is vital for the well-being of all species within an environment (Borges et al., 2022; Lewis et al., 2020; Tsafack et al., 2023; Veech, 2021). To this end, tools like the Index of Biotic Integrity (IBI) have been developed to evaluate the state of native forests (Cardoso et al., 2007). The IBI, introduced in the Azores in 2012, is a comprehensive ecological tool that assesses ecosystem health based on the presence and abundance of various species, particularly invertebrates (Soares et al., 2021). It employs multiple ecological metrics to measure habitat quality, including species richness, the presence of sensitive or tolerant species, and overall ecosystem balance. The IBI's goal is to identify trends and changes in biodiversity that reflect the health and stability of forest habitats. In the Azores, the IBI has been crucial for guiding conservation efforts and informing management practices to preserve native forest ecosystems (Chowdhury et al., 2023; Tsafack et al., 2023).

The main objective of this study was to identify and evaluate a single species or group of arthropods as bioindicators for assessing the habitat quality of native forests on Terceira Island (Azores, Portugal). Specifically, we aimed to establish correlations between arthropod populations and forest health indices (in this case, the Index of Biotic Integrity, abbreviated “IBI”) using data collected over the past 10 years from the SLAM and BALA projects. This approach seeks to provide a quicker and potentially more efficient method for assessing forest health compared to the current use of the Index of Biotic Integrity (IBI).

To accomplish this primary goal, several secondary objectives are pursued. Firstly, the study involves selecting and analysing environmental variables, such as altitude, distance to roads, and forest structure, which are known to influence habitat quality and biodiversity. This analysis is crucial for understanding the effects of these variables on species distributions and interactions within the forest ecosystem. Secondly, the research utilises long-term monitoring data from the SLAM (Sea, Land, and Air Malaise) project and the BALA (Biodiversity of Arthropods from Laurisilva of the Azores) project. These projects provide comprehensive datasets through various sampling methods, including SLAM traps and canopy beating, enabling a detailed examination of arthropod community dynamics over time.

For the statistical analysis, a Generalised Linear Mixed Model (GLMM) is employed to analyse the data from the arthropod communities. The use of GLMM is justified due to its ability to handle both fixed effects (such as environmental variables) and random effects (such as variability between sampling sites), making it particularly suitable for the hierarchical and grouped nature of the ecological data. This method allows for robust analysis of the relationships between arthropod species and IBI values while accounting for the complexity and structure of the data. By achieving these secondary objectives, the study aims to provide a robust framework for understanding and conserving the native forests of Terceira Island.

2. Materials and methods

2.1. *Study Site*

The Azorean archipelago is located in the North Atlantic between 37º-40ºN latitude and 25º-3º W longitude. The archipelago consists of nine islands distributed into three main groups: Occidental (Corvo and Flores), Central (Faial, Pico, São Jorge, Graciosa, and Terceira) and Oriental (São Miguel and Santa Maria). The Azores have a mild climate year-round, with an average annual temperature of 17ºC (Nunes et al., 2015) and high levels of humidity, up to 95% in higher-altitude native forests (Santos et al., 2004).

Terceira Island (Figure 2) exhibits a roughly circular geometry, encompassing an area of 402 km². It harbours the most extensive region of native pristine forests within the Azores, comprising five principal fragments distributed throughout the island (Triantis et al., 2010). According to the latest studies by Triantis et al. (2010), these forest remnants account for merely 5.8% of the island's total area.

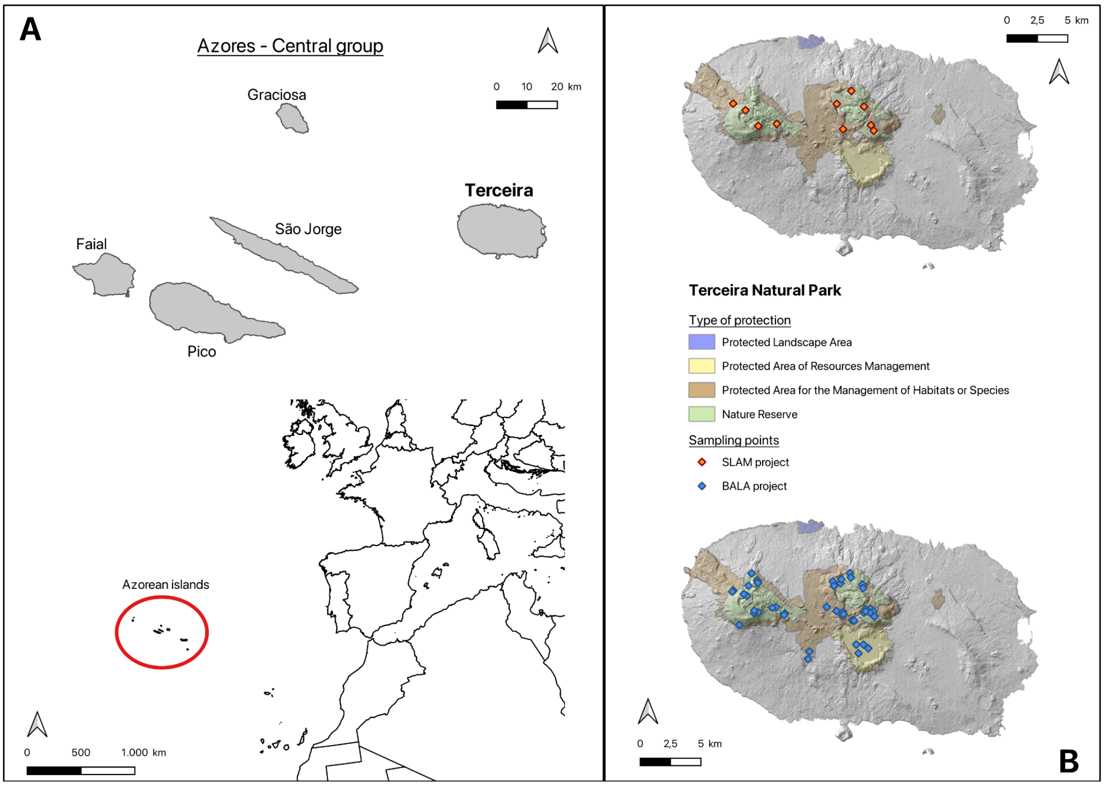

Figure 2. Study area and sampling design: (A) Terceira location in the Azorean archipelago; (B) Terceira protected areas classified by the type of protection with the sampling points. Image created by self-authorship.

The current land cover (Figure 3) is primarily composed of intensively managed pastures and fragments of exotic forests. These exotic forests include plantations of *Cryptomeria japonica* (Cupressaceae) for forestry purposes and patches of the invasive *Pittosporum undulatum* (Pittosporaceae). Native forests, characterized by evergreen vegetation, are dominated by endemic tree species such as *Juniperus brevifolia*, *Laurus azorica*, and *Erica azorica*, along with the shrub *Vaccinium cylindraceum* (Elias et al., 2016; Nunes et al., 2015; Tsafack et al., 2022). Historically, the most prevalent forest type was Laurisilva, consisting of lowland and submontane forests with tall trees. However, it is now confined to patches at elevations between 500 and 700 meters (Elias et al., 2016). The dominant forest type today occupies less than 5% of its original extent and is primarily composed of *Juniperus–Ilex* forests and *Juniperus* woodlands (Elias et al., 2016).

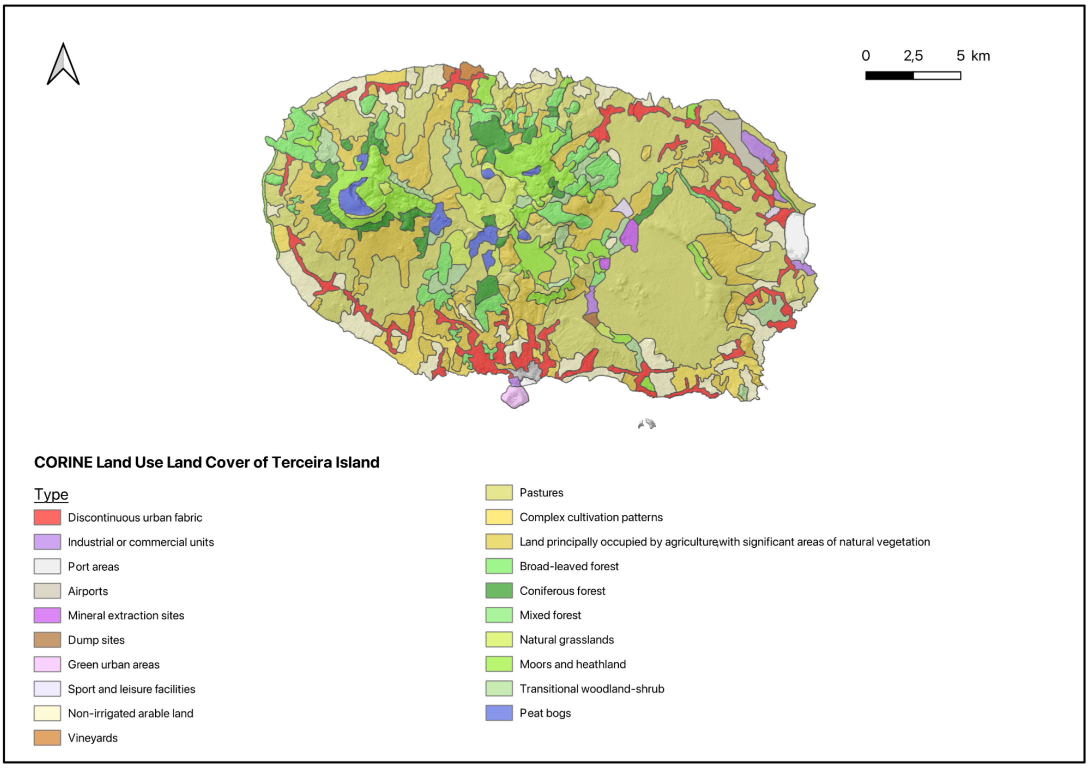

Figure 3. CORINE land use and land cover of Terceira Island. Each colour represents a different type of use or cover. Image created by self-authorship.

2.2. Data Collection

The data used for this study comes from two distinct databases: the SLAM project and the BALA project. The SLAM project (Sea, Land, and Air Malaise) is a long-term ecological study initiated in 2012, which monitors arthropod populations in the natural forests of the Azores using specialised SLAM traps (Figure 1) (Borges et al., 2022; Lamelas-Lopez et al., 2023). These traps are designed to capture a wide variety of arthropod species, providing comprehensive data on community dynamics over time.

Complementing the SLAM project, the Biodiversity of Arthropods from Laurisilva of the Azores (BALA) project, which started in 1997, focuses on the biodiversity of arthropods in the Laurisilva forests across various Azorean islands (Pozsgai et al., 2024). The BALA project employs standardised sampling methods, such as canopy beating and ground trapping, to gather detailed data on arthropod populations and their interactions within these unique forest ecosystems.

Together, these datasets offer a robust foundation for analysing the relationship between arthropod communities and forest health indices allowing us to cover the entire range of species within the native forest, from the ground to the canopy.

In addition to the databases utilised for the relevant analyses, achieving the primary objective necessitates the selection and examination of a series of environmental variables, which are well-documented for their potential impact on habitat quality, biodiversity, species distribution, and ecosystem interactions. Accordingly, we have selected several environmental variables for analysis, including altitude, distance to roads, and forest structure. These variables were chosen due to their known influence on habitat quality and biodiversity. Altitude affects temperature and humidity, which, in turn, impact species distributions and interactions (Nogués-Bravo et al., 2008). The distance to roads is pertinent as roads can fragment habitats and facilitate the spread of invasive species (Fahrig et al., 2009). Forest structure, encompassing canopy cover and understory density, affects microhabitat availability and resource distribution for arthropods (Gardner et al., 2007). By analysing these variables, we aim to understand the complex relationships between environmental factors and arthropod community dynamics, ultimately contributing to more effective conservation strategies for the native forests of the Azores.

2.2.1. SLAM Project Data

To address the main objective of finding a bioindicator species of forest health, we utilised data collected since July 2012, which involved the establishment of ten plots (50m × 50m) in the native forest (Figure 2). Each plot is equipped with a SLAM (Sea, Land, and Air Malaise) trap for long-term monitoring of arthropods (Figure 1). These passive flight-intercept traps measure 110 × 110 × 110cm and are capable of capturing arthropods from all directions (Costa & Borges, 2021). It consists of a rope-based mounting system capable of floating on water, standing on the ground or being suspended. It has four cross baffles, with the ease of collecting flying insects from the four sites making wind direction and sunlight less of a concern. A central white mesh funnels the individuals to a sampling bottle at the top of the trap where sampling bottles contain propylene glycol as a preservative. Malaise traps are useful for monitoring whether a species is present or absent (and its relative abundance) being simple, working passively and easily to operate (Tsafack, Pozsgai, et al., 2023). Previous studies have noted that they predominantly capture the most mobile segments of the invertebrate fauna (Borges et al., 2020; Matthews et al., 2019). Interestingly, despite their design as interception traps, SLAM traps have also captured a variety of flightless arthropod taxa. This expanded sampling scope is attributed to their installation: ground positioning, the complex structural environment of the native forest, and the use of the traps for dispersal (Figure 1). In this study, extending the sampling range could introduce a consistent bias. However, since the SLAM traps were set up the same way throughout the sampling period, the chances of catching different species likely stayed consistent over time. Samples were collected seasonally, with collection bottles replaced quarterly (March, June, September, and December) every sampling year.

Arthropods were initially sorted into morphospecies by parataxonomists students and subsequently identified to species level by one of the collaborators (Paulo A. V. Borges). To expedite this process, identification was concentrated on target groups: Diplopoda (Chordeumatida, Julida), Chilopoda (Geophilomorpha, Lithobiomorpha, Scolopendromorpha), Arachnida (Araneae, Opiliones, Pseudoscorpiones), and Insecta (Blattaria, Coleoptera, Hemiptera, Microcoryphia, Neuroptera, Psocodea, Thysanoptera, Trichoptera, Hymenoptera Formicidae). Specimens were then categorised by biogeographical origin: endemic (restricted to the Azores), native non-endemic (species that arrived naturally and are distributed beyond the archipelago) and invasive (species whose original range did not include the Azores before human settlement in the 15th century), based on the latest checklist of Azorean arthropods (Borges et al., 2022). The first two categories were collectively termed native, while the latter was referred to as exotic. Total abundance was considered by species (summing adult and juvenile counts), except for biomass trends, where only adults were included due to the availability of allometric equations solely for adults. For each species, previously collected adult body size data were utilised (Brush et al., 2022; Rigal et al., 2018). All collected materials are stored at EDTP—Entomoteca Dalberto Teixeira Pombo, University of the Azores, Angra do

Heroísmo, Portugal. Detailed data and sampling methodologies (including maps) are available in Costa & Borges, (2021), Lhoumeau et al. (2022a) and Lhoumeau et al. (2022b).

2.2.2. BALA Project Data

The dataset from the Biodiversity of Arthropods of the Laurisilva of the Azores, known also as BALA, originates from several initiatives employing a consistent sampling protocol for collecting arthropods across the Azorean archipelago. The BALA project was initiated in 1997 to conduct a comprehensive survey of Azorean invertebrate fauna, with a particular focus on endemic species (Borges et al., 2005; Pozsgai et al., 2024; Ribeiro et al., 2005). Over the subsequent two decades, the project expanded into the longest-running monitoring effort of the changes in the Azorean biota. Its extensive temporal and spatial scope made the BALA dataset ideal for testing macroecological and biogeographical hypotheses (Borges et al., 2011; Triantis et al., 2010), contributing to clarifying the potential occurrence of an “insect decline” in the Azores and identifying the spatial and temporal invasion patterns of exotic arthropod species (Borges et al., 2020). The project is coordinated by the Azorean Biodiversity Group (cE3c - Centre for Ecology, Evolution and Environmental Changes), based at the University of the Azores in Angra do Heroísmo, Terceira.

The core sampling, organised into three consecutive phases, repeatedly sampled the same 30 sites. During the initial sampling round (1997-2004, referred to as BALA 1), a total of 100 sites within 18 forest fragments were surveyed through the collective efforts of several projects. In subsequent rounds (2010-2012 for BALA 2 (Borges et al., 2016) and 2021-2022 for BALA 3), only 30 core sites within 15 fragments were resampled (Figure 2).

Each site was sampled for soil fauna along a 150m transect, with 30 pitfall traps placed at 5m intervals. Fifteen traps were filled with ethylene glycol, while the remaining 15 contained Turquin’s solution (10g chloral hydrate, 5 ml formalin, 5 ml acetic acid, and 1L dark beer) (Turquin., 1973). The traps were left in situ for two weeks (14 nights) of continuous operation. For canopy-dwelling arthropods (data used in this thesis) ten samples were collected per each of the three most common native tree species using a beating technique. The study focused on endemic species such as *Juniperus brevifolia* (Cupressaceae), *Erica azorica* (Ericaceae), *Ilex azorica* (Aquifoliaceae), *Laurus azorica* (Lauraceae), and *Vaccinium cylindraceum* (Ericaceae). Trees were randomly selected within a 5m radius from the pitfall trap line and beaten five times at a height of approximately 1.5–2m. Sampling was conducted from July to September, coinciding with peak arthropod activity, and canopy samples were collected exclusively during dry and warm weather conditions (Borges et al., 2005; Gaspar et al., 2008; Ribeiro et al., 2005).

2.3. *Data Selection*

Each project has its criteria for data selection, resulting in slight differences between both. Table 1 specifies the criteria applied for each database and shows the differences between them.

Table 1. Comparison of the criterion applied to select the data from the samples of SLAM and BALA project database.

Data selection criterium		
Criteria	*SLAM project*	*BALA project*
Inclusion criterion	Morphospecies with total abundance < 100 over 10 years excluded	**Abundance criterion**: Species with > 40 individuals included
		Dominance criterion: Species appearing in ≥ 50% sampling events are considered dominant
Sampling data used	All data including morphospecies sampled	Canopy data was exclusively utilised; pitfall trap data excluded
Rationale	Focuses on morphospecies with substantial abundance and consistent presence	Ensures inclusion of species with significant abundance and dominance in the canopy context, aligning with thesis objectives

2.4. *Index of Biotic Integrity (IBI)*

As explained above, the Index of Biotic integrity (IBI) was introduced by Cardoso et al. (2007) to assess the health of ecosystems by evaluating the condition of biological communities, such as arthropods, in response to human disturbances. The calculation of the IBI involves several steps:

2.4.1. *Selecting data sets*

The first step is to identify a set of taxonomic and ecological metrics that are sensitive to human disturbances. In the study of Cardoso et al. (2007) a total of 118 potential metrics were considered, including measures like species richness, abundance, diversity indices and percentages of different ecological and taxonomic groups.

2.4.2. Determining reference sites

Metrics were tested for their ability to differentiate between disturbed and undisturbed sites using statistical tests (Mann-Whitney U test). Only those metrics that consistently showed significant differences and followed the same trend (either consistently higher or lower in disturbed sites) were retained.

To ensure the selected metrics are not redundant, a Spearman rank correlation analysis was conducted. Metrics with a correlation coefficient (r) greater than 0.8 were considered redundant. Redundant metrics were removed to avoid over-representation of any particular ecological attribute.

2.4.3. Standardising and scoring the selected parameters

The selected metrics need to be standardised to be unitless and comparable. This involves dividing the range of values for each metric into three categories. The third that represents the most disturbed sites receives a score of 0, the middle third receives a score of 1, and the third that represents the most pristine sites receives a score of 2.

Finally, the candidate parameters for the calculation of the IBI include seven taxonomical and ecological parameters of arthropod communities (endemic species richness (1); predator abundance (2); predator species richness (3); native non-endemic species richness (4); saprophagous species richness (5); introduced abundance (6) and herbivore abundance(7)).

2.4.4. Calculation of the final IBI value

Although Cardoso et al. (2007) did not provide a specific formula, the final value of the Index of Biotic Integrity (IBI) for a site can potentially range between 0 and 2n, where "n" represents the number of metrics used, in this case ranging from 0 to 14. Sites with poor biological integrity will have an IBI value lower than 5, sites with moderate integrity will have an IBI value between 5 and 10, and sites with good biological integrity will have an IBI value greater than 10 (Tsafack et al., 2023). The calculated IBI values can then be used to compare the biotic integrity of different sites, reserves, or regions.

2.5. Construction of IBI-SLAM and IBI-Canopy

While the developed Index of Biotic Integrity (IBI) exhibits robustness and reliability in assessing the biological integrity of forest sites, it possesses a notable limitation: its applicability is confined to epigean arthropod communities, thus primarily encompassing ground-dwelling species (Cardoso et al., 2007).

The IBI-SLAM and IBI-Canopy indices were more recently designed to reduce bias in assessing forest habitats in the Azores by targeting specific arthropod communities in different strata (Tsafack et al., 2023a). The primary IBI served as the foundation for calculating these indices, ensuring a standardised approach to evaluate ecological integrity across diverse sampling methods.

While the IBI-SLAM focuses on the soil-litter arthropod community and the understory, the IBI-canopy evaluates the canopy layer. This approach ensures a comprehensive evaluation of forest biodiversity and ecosystem health by accounting for both ground-dwelling and canopy-dwelling arthropods. By considering multiple strata and associated arthropod communities, these indices provide a more holistic perspective on forest ecosystem integrity (Cardoso et al., 2007; Tsafack et al., 2023).

The calculation of IBI-SLAM and IBI-Canopy differs as they target distinct arthropod communities. IBI-SLAM is based on data from SLAM traps, which capture a mixed community of arthropods from 14 native and 19 disturbed forest sites, with sampling conducted in summer 2019. In contrast, IBI-Canopy focuses on canopy-dwelling species collected through the beating technique across 24 native and 14 disturbed forest sites during the same period (Tsafack et al., 2023).

To establish reference sites, Principal Component Analysis (PCA) was used to analyse arthropod abundances, identifying and excluding outlier sites that did not represent the typical conditions of native or disturbed forests. Parameters sensitive to environmental disturbance were selected, with 16 candidates for IBI-SLAM and 14 for IBI-Canopy. These parameters were then tested using Generalised Linear Modelling (GLM) to assess their ability to distinguish between native and disturbed forests.

Finally, the selected parameters were standardised and scored. Following the method by Cardoso et al. (2007), parameter values were ranked and divided into three ranges, each assigned a score of 0, 1, or 2, based on their association with either native or disturbed sites. The sum of these scores produced the final IBI value, ranging from 0 to 14, where values below 5 indicated poor biological integrity, 5 to 10 indicated moderate integrity, and above 10 indicated good integrity.

We utilised both IBI-SLAM and IBI-Canopy (for the SLAM and BALA project, respectively) to compare them with various explanatory variables to determine if any relationship exists. Additionally, we examined whether certain arthropod species in the native forest are also related to these variables.

2.6. Data Analysis

The statistical analyses were conducted using the R environment (R Core Team, 2024), with Generalised Linear Mixed Models (GLMM) applied using the ‘glmr’ function from the ‘lme4’ package (Bates et al., 2014) for both the SLAM and BALA databases. In order to carry out the main objective we have explored the relationship between specific environmental variables and the biotic integrity of forest ecosystems. In this context, the dependent variables (Y) are the IBI (Index of Biotic Integrity) values, which serve as comprehensive indicators of the ecosystem's health at various study sites, encapsulating the overall biological condition of the habitat.

The independent variables (X) include a variety of environmental factors which encompass altitude, proximity to roads, forest structure, and the presence and abundance of specific arthropod species, which serve as biotic indicators. These variables were meticulously chosen by the bibliographic research and discussed with the research group as they represent key elements that could directly or indirectly affect the ecological balance and integrity of the forest environments studied. By modelling the relationship between these independent variables and the IBI values, the analysis aims to quantify the impact of each environmental factor on the ecosystem's overall health, providing insights into the drivers of biodiversity and habitat quality in these forested areas.

The analyses also incorporated random effects to account for variability in the dependent variable (IBI) that is not explained by the fixed effects. In the SLAM database analysis, random effects included site identity and spatial location within forest fragments, capturing unexplained variability across different sites. In contrast, the BALA database analysis included random effects that accounted for temporal variations or other ecological factors specific to that dataset. A Poisson distribution was employed for the count data, appropriate for ensuring non-negative integer values, with a logarithmic link function implemented to maintain the positivity of the model’s predictions. The significance of the models was assessed using a 95% confidence level.

One key environmental factor investigated was habitat quality, as studies like those by Liu et al. (2022) and Wang & Cheng (2022) have indicated a correlation between improved habitat quality and altitude. Another important consideration is the 'edge effect,' a phenomenon introduced by Tsafack et al. (2023b), which describes changes in species composition, abundance, and ecological processes at the boundary between two different habitats, known as an ecotone. This transition zone experiences unique environmental conditions, leading to distinct ecological dynamics compared to the habitat interiors. The edge effect is typically quantified by comparing ecological parameters between edge and interior habitats through transects perpendicular to the habitat boundary (Borges et al., 2005). Understanding the edge effect is crucial for biodiversity conservation, particularly in fragmented landscapes, as it informs effective conservation strategies in response to human activities like deforestation.

The analysis also considered the biogeographic origin of species within the insect community. Island habitats, which are often highly fragmented with a high circumference-to-area ratio (Fernández-Palacios et al., 2021), exhibit a close relationship between species' biogeographic origin and the edge effect, particularly in island ecosystems (Tsafack et al., 2023b). Species were classified as endemic (restricted to a specific island or archipelago), native non-endemic (naturally occurring on the islands and elsewhere), or invasive (introduced by humans). Invasive species may thrive in edge habitats due to their adaptability to environmental changes, whereas indigenous species (endemic and native non-endemic) tend to have more specific habitat requirements (Borges et al., 2020).

2.6.1. SLAM project database GLMM model

For the SLAM project database, we used the following formula to fit into the GLMM model:

$$IBI \sim MF117 \; + \; ... \; + \; MF927 \; + \; (1 \mid SRTM\_elevation) \; + \; (1 \mid edge\_dist) \qquad \text{(eq. 1)}$$

In this formula, IBI is the dependent variable we want to analyse with our random effects, which are:

- **SRTM_elevation**: This refers to the altitude of the study area
- **edge_dist**: This represents the distance to the nearest road or the border of the habitat where the sample point is located; more detailed in Tsafack et al. (2023b) publication.
- The term "**MF**" is an abbreviation for "morphospecies" and is used to represent each species in our database. Each individual species is designated by "MF" followed by one, two or three numbers (e.g., MF6, MF89 or MF927), which serve as the fixed effects in the equation (for details on all individuals used, refer to Appendix A - Table 5).

2.6.2. BALA project database GLMM model

For the BALA project database, we used the same analysis as mentioned above, with slight modifications to the formula (see the results section for the reasons for the modification).

$$IBI \sim MF117 \; + \; ... \; + \; MF927 \; + \; (1 \mid site\_code) \; + \; (1 \mid project) \qquad \text{(eq. 2)}$$

In this formula, as before, IBI is also the dependent variable we want to analyse with our random effects, which are:

- **site_code**: Represents the spatial distribution of the sampling points
- **project**: Accounts for the different phases of the BALA project (BALA 1, BALA 2 and BALA 3).
- The term "**MF**" is an abbreviation for "morphospecies" and is used to represent each species in our database. Each individual species is designated by "MF" followed by one, two or three numbers (e.g., MF6, MF89 or MF927), which serve as the fixed effects in the equation (for details on all individuals used, refer to Appendix A - Table 5).

3. Results

3.1. SLAM Project Data

3.1.1. GLMM – Random effects

The results for the GLMM of the SLAM project database for the random effects (Table 2) show the variance and standard deviation for the groups "SRTM_elevation" and "edge_dist". The variance and standard deviation for "SRTM_elevation" are both 0, indicating no contribution to the model's variability. The random effect for "edge_dist" has a very small variance of 4.074e-19 and a standard deviation of 6.383e-10, also suggesting a negligible contribution to the model's variability.

Table 2. Variance and standard deviation of the random effects of the model for the "SRTM_elevation" and "edge_dist" groups for the SLAM project database.

Random effect			
Groups	*Name*	*Variance*	*Std.Dev*
SRTM_elevation	(Intercept)	0.000e+00	0.000e+00
edge_dist	(Intercept)	4.074e-19	6.383e-10

3.1.2. GLMM – Fixed effects

The findings from the GLMM analysis of the SLAM project database, focusing on the fixed effects, are detailed in Table 3. This table provides the coefficient estimates, standard errors, Z-values, and P-values for each fixed effect. Notably, the intercept, which represents the baseline level of the dependent variable when all fixed effects are zero, significantly differs from zero (estimate = 2.336, P < 2e-16), underscoring its fundamental role in the model. The fixed effects, denoted by the MF terms, elucidate the unique impacts of various species on the dependent variable. Of these effects, only MF137 (*Pinalitus oromii*) emerges as statistically significant (estimate = 0.00587, P-value = 0.0411), indicating a noticeable positive relationship with the dependent variable. Conversely, the majority of fixed effects lack statistical significance, suggesting that their influences on the model are negligible at the 95% confidence level. Although MF7 (*Cixius azoterceirae*) presents a P-value of 0.0715, this result does not reach statistical significance under the conventional threshold of 0.05. Therefore, any interpretation of this result should be approached with caution.

Table 3. Coefficient estimates, standard errors, Z-value and P-value of fixed effects in the GLMM model for SLAM project database. Asterisks indicate statistical significance of coefficients (0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

Fixed effect				
	Estimation	*Std. error*	*Z-value*	*P-value*
(Intercept)	2.336	0.039	60.499	< 2e-16 ***
MF191	- 0.00052	0.00753	- 0.069	0.9451
MF439	- 0.00610	0.00426	- 1.433	0.1520
MF59	- 0.00632	0.00845	- 0.748	0.4543
MF476	0.00564	0.01614	0.349	0.7268
MF198	0.00027	0.01358	0.020	0.9844
MF27	- 0.00330	0.01044	- 0.316	0.7522
MF179	0.00827	0.01940	0.426	0.6700
MF6	- 0.00148	0.00192	- 0.770	0.4413
MF195	- 0.00088	0.00299	- 0.295	0.7682
MF117	- 0.00546	0.01545	- 0.353	0.7238
MF167	- 0.02349	0.02924	- 0.803	0.4218
MF13	- 0.01194	0.01835	- 0.651	0.5151
MF200	0.00411	0.00683	0.602	0.5474
MF134	0.00136	0.00798	0.170	0.8647
MF465	- 0.02538	0.02664	- 0.952	0.3409
MF370	- 0.00349	0.00314	- 1.111	0.2666
MF184	- 0.02256	0.02145	- 1.052	0.2929
MF144	- 0.00149	0.00148	- 1.008	0.3136
MF121	-0.02126	0.01639	- 1.297	0.1945
MF46	0.02289	0.02433	0.941	0.3468
MF124	0.00013	0.00390	0.034	0.9733
MF7	0.00107	0.00059	1.802	0.0715 .
MF44	0.00002	0.00564	0.003	0.9974
MF478	- 0.02149	0.02206	- 0.974	0.3300
MF257	- 0.02019	0.01802	- 1.120	0.2626
MF141	0.00252	0.00592	0.426	0.6703
MF57	0.02265	0.03537	0.640	0.5219
MF78	0.00087	0.01252	0.069	0.9448
MF312	0.00034	0.00790	0.044	0.9652
MF21	0.00734	0.02428	0.302	0.7625

MF89	- 0.00240	0.02641	- 0.091	0.9275
MF557	0.01044	0.02405	0.434	0.6643
MF181	0.00289	0.00537	0.538	0.5903
MF5	0.00338	0.00462	0.731	0.4646
MF102	0.00827	0.01268	0.652	0.5143
MF39	0.00465	0.02377	0.195	0.8450
MF137	0.00587	0.00288	2.043	0.0411 *
MF421	0.01483	0.02697	0.550	0.5824
MF9	- 0.00498	0.03083	- 0.161	0.8718

3.2. BALA Project Data

3.2.1. GLMM – Random effects

The results for the GLMM of the BALA project database for the random effects (Table 4) show the variance and standard deviation for the groups "site_code " and "project ". The random effect associated with "site_code" exhibits a variance and standard deviation of 0, indicating negligible variability in the model attributable to this grouping factor. Similarly, the random effect associated with "project" demonstrates an extremely small variance (6.944e-19) and standard deviation (8.333e-10), further suggesting a minimal contribution to the model's variability.

Table 4. Variance and standard deviation of the random effects of the model for the “project” and “site_code” groups for the BALA project database.

Random effect			
Groups	*Name*	*Variance*	*Std.Dev*
site_code	(Intercept)	0.000e+00	0.000e+00
project	(Intercept)	6.944e-19	8.333e-10

3.2.2. GLMM – Fixed effects

The GLMM model applied to the BALA project database, as detailed in Table 5, unveils significant insights into the fixed effects impacting the dependent variable. Notably, the intercept, indicating the baseline level of the dependent variable, significantly differs from zero (estimate = 1.9598, p < 2e-16), underscoring its pivotal role. While the coefficient for MF9 (*Ommatoiulus moreleti*) demonstrates statistical significance (estimate = -0.0618, p-value = 0.0197), suggesting a discernible impact, most other fixed effects lack statistical significance at α = 0.05, implying minimal contributions. Some effects, such as MF144 (*Trigoniophthalmus* *borgesi*), show marginal importance with a P-value of 0.0621, which is not statistically significant. Therefore, any interpretation of this result should be approached with caution.

Table 5. Coefficient estimates, standard errors, Z-value and P-value of fixed effects in the GLMM model for BALA project database. Asterisks indicate statistical significance of coefficients (0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

Fixed effect				
	Estimation	*Std. error*	*Z-value*	*P-value*
(Intercept)	1.9598	0.1557	12.578	< 2e-16 ***
MF117	0.0003	0.0011	0.224	0.8228
MF121	- 0.0025	0.0058	- 0.422	0.6733
MF124	- 0.0019	0.0022	- 0.830	0.4063
MF134	0.0002	0.0019	0.088	0.9299
MF137	- 0.0005	0.0068	-0.078	0.9377
MF144	0.0075	0.0040	1.866	0.0621 .
MF176	- 0.0034	0.0045	- 0.760	0.4475
MF179	0.0057	0.0031	1.877	0.0605 .
MF181	0.0010	0.0008	1.209	0.2266
MF19	0.0004	0.0006	0.717	0.4733
MF191	-0.0059	0.0097	- 0.604	0.5457
MF195	-0.0005	0.0009	-0.522	0.6020
MF198	-0.0059	0.0076	-0.775	0.4382
MF200	-0.0098	0.0157	-0.625	0.5318
MF208	-0.0216	0.0306	-0.705	0.4806
MF21	-0.0276	0.0273	-1.011	0.3120
MF3	0.0080	0.0054	1.472	0.1409
MF312	-0.0058	0.0084	-0.696	0.4863
MF370	0.0063	0.0038	1.645	0.0999 .
MF414	0.0050	0.0063	0.784	0.4331
MF42	0.0148	0.0195	0.757	0.4493
MF44	-0.0007	0.0007	-1.057	0.2904
MF440	0.0019	0.0029	0.664	0.5064
MF5	0.0001	0.0011	0.102	0.9185
MF59	-0.0030	0.0018	-1.612	0.1070
MF7	0.0012	0.0012	1.052	0.2930
MF9	-0.0618	0.0265	-2.333	0.0197 *

MF90	0.0017	0.0027	0.621	0.5348
MF927	-0.0030	0.0056	-0.547	0.5844

3.3. Species of concern

Figure 4 displays two scatter plots that examine the relationship between the Index of Biotic Integrity (IBI) and the abundance of two different species (Figure 5), highlighting significant trends.

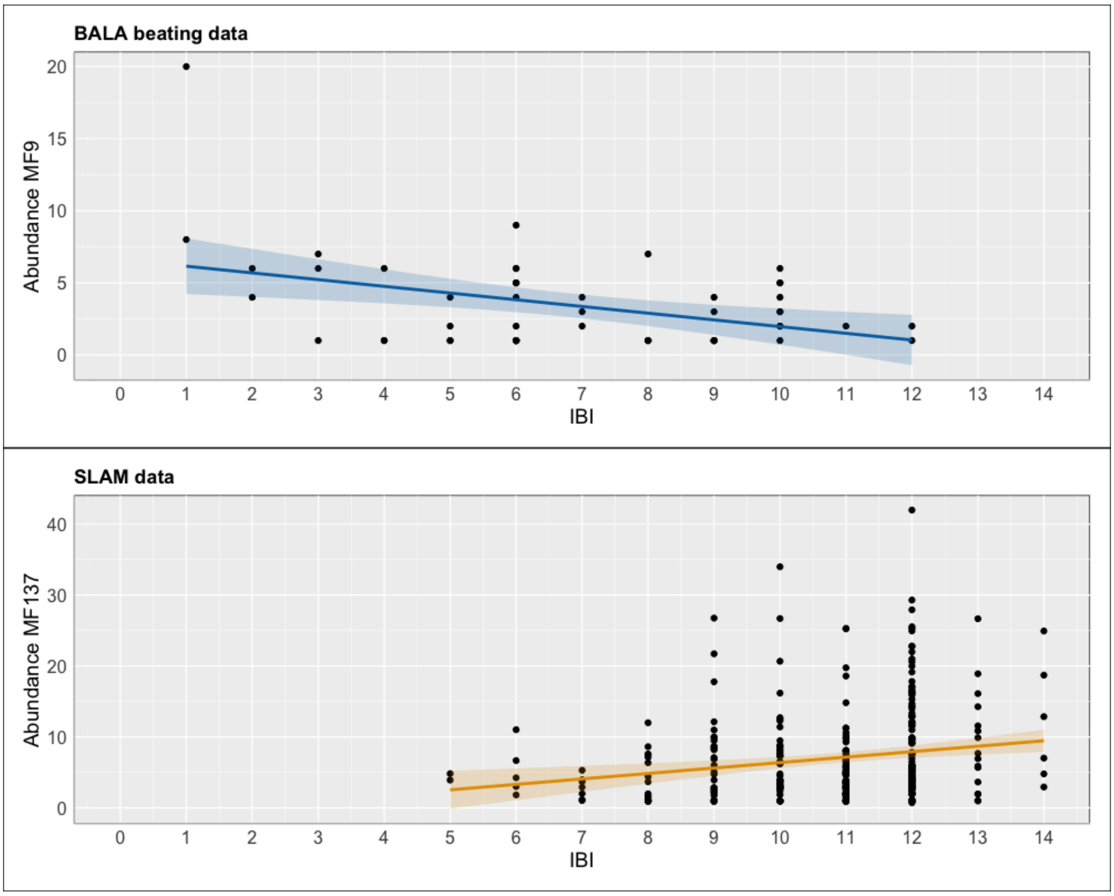

Figure 4. Scatterplot showing the relationship between arthropod abundance and Index of Biotic Integrity (IBI) in BALA and SLAM projects. The top panel (BALA data) shows a negative correlation between the abundance of MF9 (*Ommatoiulus moreleti*) and IBI, with the shaded blue area indicating the 95% confidence interval. The bottom panel (SLAM data) depicts a positive correlation between the abundance of MF137 (*Pinalitus oromii*) and IBI, with the shaded orange area representing the 95% confidence interval.

The top panel presents the scatter plot for *Ommatoiulus moreleti* within the BALA database. This plot similarly depicts individual data points showing the observed abundance of *Ommatoiulus moreleti* against corresponding IBI values. The blue regression line here indicates a negative trend, with a decrease in the abundance of *Ommatoiulus moreleti* as IBI values increase. The shaded area around the regression line represents the 95% confidence interval, indicating the likely range of the true regression line. The spread of points around the regression line suggests variability, yet the overall downward trend is evident.

In the bottom panel, the scatter plot shows the abundance of *Pinalitus oromii* across different IBI values within the SLAM database. Each point represents the observed abundance of *Pinalitus oromii* at a given IBI value. The orange regression line, accompanied by a shaded 95% confidence interval, indicates a slight positive trend. The distribution of points, particularly the clustering at higher IBI values, underscores this positive relationship, despite some variability in abundance.

It is important to note that these two graphs cannot be directly compared due to the different sampling methods used. The SLAM database was collected using SLAM traps, while the BALA database was obtained through canopy beating. These methodological differences influence the type and quantity of data collected, and therefore, the results should be interpreted independently rather than comparatively.

Figure 5. Images of key arthropod species analysed in this study. On the left is *Pinalitus oromii*, an endemic species showing a positive correlation with higher Index of Biotic Integrity (IBI) values, suggesting its utility as an indicator of pristine forest conditions. On the right is *Ommatoiulus moreleti*, an invasive species exhibiting a negative correlation with IBI values, indicating its presence in disturbed habitats. These species were the only ones to yield significant results in the analyses of this thesis. Images from Enésima Mendonça (ABG-cE3c) and Pedro Cardoso (ABG-cE3c) extracted from Azores BioPortal (ABP) - https://azoresbioportal.uac.pt/

4. Discussion

While using indicator taxa as proxies for biodiversity is generally considered unreliable, they can still be effective within specific ecosystem and geographic boundaries. Indicators prove valuable in pinpointing ecological traits or tracking the outcomes of habitat management practices, especially in assessing progress in restoration efforts (Gerlach et al., 2013). The main aim of our study was to examine the potential of a single species or group of arthropods as bioindicators to assess different states of forest integrity within Azorean forest fragments. Existing studies, such as those by Legal et al. (2020), Menta & Remelli (2020), Pearce et al. (2006) and Spiller et al. (2018) have explored the use of bioindicators for various purposes in different regions worldwide, but there remains a critical need for more specific information on species that can be directly utilised in the field across the diverse islands of the Azorean archipelago and beyond. This gap highlights the necessity for localised research to identify and validate bioindicators that are relevant to the unique ecological contexts of these islands, thereby enhancing the effectiveness of biodiversity monitoring and conservation strategies (Cooper et al., 2009; Kamarudin et al., 2012; Lachs et al., 2019).

The hypothesis that arthropod populations can serve as effective bioindicators of forest health is supported by the data analysed. This study suggests that two species, the Azorean endemic true bug species *Pinalitus oromii* and the invasive millipede species *Ommatoiulus moreleti,* can be used as indicators to determine the status of native forests on Terceira Island.

Ommatoiulus moreleti, commonly referred to as the Portuguese millipede, is a notable example of an invasive species that has established itself in various regions outside its native range, as in the Azores archipelago. Originally from the Iberian Peninsula, *O. moreleti* has successfully colonised diverse environments, often considered a pest in some countries as reported by Douglas et al. (2019). It thrives in moist, sheltered environments such as leaf litter, under logs, and within soil rich in organic matter, even occurring in both Azorean urban and underground environments. Native forest habitats provide the ideal conditions for its survival and reproduction, enabling it to form dense populations. The millipede's ability to adapt to a variety of disturbed habitats, including agricultural lands and urban areas, further facilitates its spread (Gilgado et al., 2022). The species is detritivorous, feeding primarily on decaying plant material, which plays a role in nutrient cycling within ecosystems (Douglas et al., 2017). This species belongs to the class Diplopoda, characterised by a cylindrical body composed of numerous segments, each bearing two pairs of legs. Adults can reach lengths of up to 45 mm and display a dark brown to black colouration, which can aid in their identification (Mesibov, 2012). The species is also very common and spreading in Canary Islands (Arndt et al., 2008), which implies that it can potentially also be used as an indicator in other Macaronesian islands.

Millipedes are a key group of terrestrial arthropods that contribute significantly to the decomposition of organic matter and serve as potential bioindicator taxa due to their low tolerance and high sensitivity to habitat changes (Masse & Paul Serge, 2016). Also, they have been used mainly as indicators of habitat characteristics (Kappes et al., 2009; Uys et al., 2010) and the effects of management (Halaj et al., 2009) and restoration (Snyder & Hendrix, 2008).

Pinalitus oromii, a true bug from the family Miridae (Insecta, Hemiptera), represents a critical component of the native biodiversity in the Azores. Unlike the invasive *O. moreleti*, *P. oromii* is endemic to the Azores archipelago, highlighting its importance for conservation and ecological studies. It inhabits the native forests and undisturbed habitats on the Azores islands, where it is closely associated with the local flora. The species utilises its piercing-sucking mouthparts to feed on plant sap, playing a role in the regulation of plant community dynamics. Its presence in native forests makes it a valuable indicator of ecological integrity and forest health (Cardoso et al., 2009). *Pinalitus oromii* belongs to the class Insecta, order Hemiptera, and family Miridae. It is relatively small, with adults typically ranging from 3 to 5 mm in length. The species is identified by its distinctive body shape and colouration, which aids in its recognition and classification. As an endemic species, is it crucial for maintaining the ecological balance within the Azorean forests (Borges et al., 2022).

When discussing the order Hemiptera, commonly known as bugs, their use as bioindicators in terrestrial environments has been limited, despite the potential of certain taxa. This order is highly diverse, encompassing a wide range of ecological forms, and is abundant in nearly every type of habitat. Bugs are relatively easy to identify at the morphospecies level, although some families pose identification challenges (Gerlach et al., 2013; Jana et al., 2006). As ecological indicators, bugs have been employed to monitor pollution levels and assess the impacts of habitat drainage (Skern et al., 2010). Additionally, bugs have been included in studies evaluating habitat restoration progress post-mining activities (Orabi et al., 2010). One of the challenges with this species is its small size, which complicates field identification and collection. Its ability to fly further exacerbates these difficulties, as it can easily escape during collection attempts. Additionally, accurate identification in the field requires magnification tools or specialised instruments due to its minute size. This challenge is not unique to our study; similar difficulties have been documented in other research. For example, Novotny et al. (2000) discuss the problems associated with sampling and identifying small, rare insect species in tropical environments, emphasising the issues arising from their size and mobility. Similarly, Pearce et al. (2006) discuss the problems associated with using ground beetles as bioindicators due to their small size and the necessity of specialised identification tools (even though it is not a species documented in this study). Additionally, studies on the monitoring and conservation of invertebrates often highlight the difficulties faced in field identification of small species and the need for appropriate tools and methods (Cardoso et al., 2011). However, *Pinalitus oromii* has a particular colour pattern that may help to be distinguished in the field after some experience.

In Figure 4 *Pinalitus oromii* shows a positive correlation with the Index of Biotic Integrity (IBI), suggesting that its presence and high abundance are indicative of healthier forest conditions. This aligns with the findings of Tsafack et al. (2023), who also identified species with strong correlations to environmental disturbances as effective bioindicators. Conversely, *Ommatoiulus moreleti* exhibited a negative relationship with the IBI (Figure 4), indicating that higher populations of this species may signal poorer forest health or increased disturbance. This finding is consistent with Cardoso et al. (2007), who emphasised the importance of identifying species that respond predictably to environmental stressors. However, the study's limitations, such as the negligible random effects of variables like altitude ("SRTM_altitude") and the distance to the nearest road ("edge_dist") suggest that future research should explore additional environmental factors that might influence arthropod populations.

The selection of sampling methods in the BALA and SLAM projects—canopy beating and SLAM traps, respectively—was guided by practicality and accessibility considerations. These methods were chosen for their ease of implementation, aiming to make them accessible for use by non-specialists, as outlined in the study by Basset et al. (2001). Beating, which involves dislodging arthropods from vegetation onto a collecting surface, is a straightforward method requiring minimal equipment (Schowalter & Chao, 2021). This makes it more feasible for non-specialists and volunteers to participate in arthropod sampling, enhancing community involvement in ecological monitoring (Missa et al., 2009). Pitfall traps, in contrast, require more setup and maintenance, which could be a deterrent for widespread use by the park rangers (Costa-Silva et al., 2019).

In evaluating the effectiveness of bioindicator species, *Ommatoiulus moreleti* (BALA MF9) emerged as a more advantageous candidate compared to *Pinalitus oromii* (BALA MF137) for several reasons. Firstly, the ease of sampling *O. moreleti* makes it a more practical choice for monitoring programs. Its larger size, distinctive morphology, and broader aspect make it easily identifiable in the field, which is crucial for reliable data collection (Cardoso et al., 2011; Han et al., 2015). Additionally, the range of IBI values where *O. moreleti* is found is broader, providing a more comprehensive indicator of forest health across different conditions. This wider distribution enhances its utility as a bioindicator, as it can reflect a more extensive range of environmental states (Borges et al., 2018). Furthermore, as an invasive species, *O. moreleti* can be ethically sampled and removed from the ecosystem without conservation concerns. In contrast, *P. oromii* is an endemic species, and ethical considerations preclude its removal from the environment, limiting its use as a bioindicator (Tsafack et al., 2023).

Despite the differences in sampling methods between the SLAM and BALA projects, the consistent identification of bioindicator species across these methods underscores their robustness as indicators of forest health (Missa et al., 2009).

5. Conclusions

This study provides significant insights into the potential of arthropod communities as bioindicators for assessing forest integrity within Azorean forest fragments. By examining two specific species, *Pinalitus oromii* and *Ommatoiulus moreleti*, we have identified viable indicators that reflect different states of forest health. Our findings align with previous research and contribute to the growing body of knowledge on the use of arthropods in ecological monitoring and conservation.

Ommatoiulus moreleti, an invasive millipede, demonstrated a negative correlation with the Index of Biotic Integrity (IBI), indicating that higher populations of this species are associated with poorer forest conditions or increased disturbances. Its ease of sampling and identification, coupled with its broader range of IBI values, make it a practical and effective bioindicator for monitoring forest health. As an invasive species, its removal poses no ethical concerns, further supporting its use in conservation programs.

Conversely, *Pinalitus oromii*, an endemic true bug, exhibited a positive relationship with the IBI, suggesting that its presence and abundance are indicative of healthier forest conditions. However, ethical considerations preclude the removal of this species from the environment, limiting its application as a bioindicator despite its ecological significance.

The study's methodology, incorporating both canopy beating and SLAM traps, highlights the importance of practical and accessible sampling techniques. These methods were chosen to facilitate participation by non-specialists, ensuring broader community involvement in ecological monitoring. The negligible impact of certain random effects, such as altitude and distance to the nearest road, suggests that future research should explore additional environmental factors influencing arthropod populations.

Our research underscores the necessity for localised studies to identify and validate bioindicators relevant to specific ecological contexts, particularly in the diverse environments of the Azorean archipelago. While existing studies have explored the use of bioindicators globally, our findings emphasise the importance of region-specific research to enhance the effectiveness of biodiversity monitoring and conservation strategies.

This study contributes to the understanding of arthropod communities as bioindicators of forest health. The identification of *Ommatoiulus moreleti* and *Pinalitus oromii* as potential indicators provides a foundation for future research and conservation efforts in the Azores. By advancing our knowledge of bioindicators and refining our monitoring techniques, we can better assess and manage the biodiversity and ecological integrity of these unique forest ecosystems.

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7. Acknowledgement

Probablement aquest treball marqui el final de la meva etapa com a estudiant. Tot i que mai es deixa d'aprendre, tot camí té un final i aquest és el meu en aquesta època de la meva vida. No sempre ha estat fàcil; hi ha hagut moments en què no veia clar per on anar, moments sense cap motivació ni il·lusió, moments en què no tenia ganes de seguir endavant i volia deixar-ho tot perquè no em sentia bé amb mi mateix. Però gràcies a les persones que m'envolten, que m'estimen i m'han ajudat en aquests moments de dubte, un camí amb molta boira, on no podia veure el final, s'ha convertit en només una petita tempesta. Tot i que no sé exactament cap a on anar en el futur ni on acabaré, tinc més clar que mai que vull fer-ho amb molta il·lusió i gaudint de tot el que em queda per aprendre, descobrir i conèixer.

No tinc prou paraules per agrair a totes les persones que heu estat al meu costat quan més ho necessitava, i encara menys a tu avi. Gràcies per rebre'm sempre amb un somriure, per tot el que ens has ensenyat i ajudat, però sobretot gràcies per tot el que ens has estimat. Desitjaria que poguessis ser aquí, et trobem molt a faltar.

La felicitat no és fer el que un vol, sinó voler el que un fa.

Jean-Paul Sartre

8. Appendix A – Supplementary Data

Table S1. Association between the scientific name and the alphanumeric ending. The letters of the biogeographic origin column refer to whether the species is endemic (E), native non-endemic (NE) or invasive (I).

Alphanumeric designation	**Scientific name**	**Biogeographic origin**
MF191	*Valenzuela flavidus*	N
MF439	*Notothecta dryochares*	E
MF59	*Zetha simonyi*	N
MF476	*Monalocoris filicis*	N
MF198	*Macaroeris cata*	N
MF27	*Lithobius pilicornis pilicornis*	N
MF179	*Leucognatha acoreensis*	E
MF6	*Leiobunum blackwalli*	N
MF195	*Trioza laurisilvae*	N
MF117	*Lathys dentichelis*	N
MF167	*Kleidocerys ericae*	N
MF13	*Hoplothrips corticis*	N
MF200	*Hemerobius azoricus*	E
MF134	*Gibbaranea occidentalis*	E
MF465	*Eupteryx azorica*	E
MF370	*Elipsocus brincki*	E
MF184	*Elipsocus azoricus*	E
MF144	*Trigoniophthalmus borgesi*	E
MF121	*Ectopsocus briggsi*	I
MF46	*Drouetius borgesi borgesi*	E
MF124	*Cyphopterum adscendens*	N
MF7	*Cixius azoterceirae*	E
MF44	*Cinara juniperi*	N
MF478	*Trichopsocus clarus*	N
MF257	*Catops coracinus*	N
MF141	*Calacalles subcarinatus*	E
MF57	*Atheta aeneicollis*	???
MF78	*Anaspis proteus*	N
MF312	*Acorigone acoreensis*	E
MF21	*Tenuiphantes tenuis*	I

MF89	*Tachyporus nitidulus*	???
MF557	*Strophingia harteni*	E
MF181	*Savigniorrhipis acoreensis*	E
MF5	*Rugathodes acoreensis*	E
MF102	*Pseudophloeophagus tenax borgesi*	E
MF39	*Pisaura acoreensis*	E
MF137	*Pinalitus oromii*	E
MF421	*Walckenaeria grandis*	E
MF9	*Ommatoiulus moreleti*	I
MF3	*Xysticus cor*	N
MF42	*Nycterosea obstipata*	N
MF440	*Rhopobota naevana*	I
MF90	*Cyclophora azorensis*	E
MF927	*Cheiracanthium erraticum*	I