



Ana Carolina Pinto Duarte

**Effects of protected areas on
soil functional biodiversity in
the North of Portugal**

Universidade do Minho
Escola de Ciências





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Dissertação de Mestrado
Mestrado em
Biodiversidade, Ecologia e
Alterações Globais

Trabalho efetuado sob a orientação de:
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Effects of protected areas on soil functional biodiversity in the North of Portugal

Abstract

Soils hold the larger part of the biomass on Earth, being one of the largest reservoirs of biological diversity, and providing a variety of ecosystem services crucial for the integrity of above-ground biodiversity and human well-being. However, nature conservation policy is only concerned with aboveground terrestrial species and soil biodiversity has been largely ignored, resulting in a lack of information about the conservation status of most soil organisms and about the effects that nature conservation policies have on soil systems. Forests in Northern Portugal are characterized by a remarkable diversity of ecosystems, both natural and semi-natural, as well as anthropogenic, and provide habitat for an enormous diversity of living organisms, including some of the rarest and most valuable elements of regional and national biological diversity. This study aims to investigate whether and to what extent protected areas increase soil biodiversity and function in forest ecosystems. Specifically, this aims i) to assess differences in soil biodiversity and function between protected and non-protected areas, across forests dominated by native or exotic species, on a regional scale; and ii) to infer on how protected areas deal with pressures and drivers of soil biodiversity and function. The study area, the North of Portugal, comprises a vast range of edaphoclimatic conditions and environmental pressures, that have been well-characterised by the SoilReCon Project (PTDC/BIA-CBI/2340/2020) through a wide sampling campaign. To ascertain levels of soil biodiversity, quality, and functioning, we employed nematode community analyses, that have been widely used for their bioindicator value. With this study we showed that native forest areas had a more nutrient-enriched soil system and a more complex community structure with a tendency for lower disturbances and, because of that, are better suited to sustain soil biodiversity and functions and should have a greater importance in nature conservation measures. Protected areas tended to positively affect soil biodiversity and function, but this effect was not significant. Among the assessed environmental variables, those that mostly influenced soil functional biodiversity were soil organic carbon, soil apparent density, soil moisture, deciduous forest cover, and pH. It then follows that conservation efforts targeted at the protection of native forests need to better consider effects on soil biodiversity and function, to provide an integrated protection of both aboveground and belowground components.

Key Words: Forest areas, Functions, Nematodes, North of Portugal, Protected areas

Efeitos das áreas protegidas na biodiversidade funcional do solo no Norte de Portugal

Resumo

Os solos detêm a maior parte da biomassa da Terra, sendo um dos maiores reservatórios de biodiversidade e fornecendo uma variedade de serviços cruciais para a biodiversidade acima do solo e bem-estar humano. No entanto, a política de conservação da natureza tem sido vocacionada apenas para a biodiversidade acima do solo, sendo a biodiversidade do solo ignorada, com a resultante falta de informação sobre o estado de conservação da maioria dos organismos do solo e sobre os efeitos que as políticas de conservação da natureza têm nos sistemas solo. As florestas do Norte de Portugal são caracterizadas por uma notável diversidade de ecossistemas, naturais, seminaturais, bem como antropogénicos, e fornecem habitat a uma enorme diversidade de organismos, incluindo alguns dos mais raros e valiosos a nível regional e nacional. Este estudo tem como objetivo investigar se e em que medida as áreas protegidas aumentam a biodiversidade e as funções do solo nos ecossistemas florestais. Especificamente, visa: i) avaliar as diferenças na biodiversidade e funções do solo entre áreas protegidas e não protegidas, em florestas nativas e exóticas; e ii) inferir como as áreas protegidas lidam com as pressões e fatores determinantes da biodiversidade e funções do solo. O Norte de Portugal compreende uma vasta gama de condições edafoclimáticas e pressões ambientais, bem caracterizadas pelo Projeto SoilReCon (PTDC/BIA-CBI/2340/2020) através de uma ampla campanha de amostragem. Para verificar os níveis de biodiversidade, qualidade e funcionamento do solo, usámos análises de comunidades de nemátodes, amplamente utilizados pelo seu valor bioindicador. Concluimos que as florestas nativas apresentavam um sistema solo mais rico em nutrientes e uma estrutura de comunidade mais complexa com tendência a menores distúrbios e, por isso, devendo ter uma maior importância para as medidas de conservação. As áreas protegidas tendiam a efeitos positivos na biodiversidade e funções do solo, mas esse efeito não foi significativo. Dentre as variáveis avaliadas, as que mais influenciaram a biodiversidade funcional do solo foram o carbono orgânico do solo, a sua densidade aparente, humidade e pH, bem como a cobertura florestal caducifolia. Os esforços de conservação direcionados à proteção de florestas nativas precisam considerar melhor os efeitos sobre a biodiversidade e funções do solo, para fornecer uma proteção integrada dos componentes acima e abaixo do solo.

Palavras-chave: Áreas Florestais, Áreas Protegidas, Funções, Nematodes, Norte de Portugal

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List of Abbreviations

MI – Maturity Index

EI – Enrichment Index

SI – Structure Index

CI – Channel Index

BI – Basal Index

IUCN – International Union for Nature Conservation

SPA – Special Protection Areas

SCI – Sites of Community Interest

ICNF – Institute for Nature Conservation and Forests

RNAP – National Network of Protected Areas

PA – Protected Areas

nonPA – Non-Protected Areas

FOR – Native Forests

EXO – Exotic Forests

CCA – Correspondence Canonical Analysis

VIF – Variance Inflation Factor

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1. INTRODUCTION

1.1 Importance of soils and soil communities for terrestrial ecosystems

Soils hold 60% to 90% of the biomass on Earth (Dobrovolskaya et al., 2015) being one of the largest reservoirs of biological diversity (Bardgett & van der Putten, 2014), and providing a variety of ecosystem services including carbon and nutrient cycling and the decomposition of organic matter, and benefits such as climate regulation and suppression of soilborne pests and diseases (Brussaard, 1997; Gessner et al., 2010; Guerra et al., 2021; Wall et al., 2015). They regulate the diversity and functioning of aboveground systems, as well as their benefits to human well-being (Bardgett & van der Putten, 2014; Guerra et al., 2021), being particularly important in forest areas for the decomposition of organic matter and carbon sequestration (Gessner et al., 2010; Ruan et al., 2005). As the substrate for the majority of human activities, such as agriculture, buildings, and transport, soils offer the necessary resources for industrial use and waste management, and have an important role in the elemental cycles, without which agriculture, its biodiversity, ecosystem goods and services to human society would be impossible (Brussaard, 1997; Bardgett & van der Putten, 2014). Soil processes are carried out and regulated by its biodiversity, which affects its physical and chemical fertility (Giller, 1996); the hydrology, aeration, and gaseous composition of the soils, essential for primary production and decomposition of organic residues and waste materials, are also influenced by soil communities (Brussaard, 1997).

Given the distinct biogeographical patterns of soil biota, degradation in its integrity will have direct and indirect effects on Earth's biodiversity: directly by causing local extirpation or global extinction of certain soil organisms, and indirectly by altering species-specific interactions between soil-dwellers and other organisms or altering ecosystem functions (Parker, 2010). Having this in mind, we cannot guarantee future aboveground biodiversity and food supply if we do not safeguard soils and their biodiversity for future generations (Guerra et al., 2021). Soil biota are sensitive to changes in their environment and, therefore, can also be used for early diagnosis of multiple ecosystem function variations (Havlicek, 2012).

1.2 Nematodes as indicators of ecosystem functions

Soil nematodes are microscopic roundworms that reside in water films surrounding soil particles. They are the most abundant animals on Earth, occurring at all trophic levels of the soil food web, and constitute a dominating component of the soil community (van den Hoogen et al., 2019). Nematodes can be

classified in functional groups based on their morphology and feeding habits, with most soils including bacterivores, fungivores, herbivores, omnivores and predators (Yeates et al., 1993). Their trophic group can be easily inferred but their mouth and upper digestive system structure: bacterivores ingest bacteria and have a cylindrical and narrow oral cavity; fungivores have a fine stylet to feed on fungal hyphae; herbivores have a stylet to pierce plant cells; predators have a large oral cavity with teeth, denticles and/or sharp plates with which they feed on other soil organisms including other nematodes; and omnivores, that bear an odontostyle, can functionally act like bacterivores, fungivores, herbivores and predators (Goulart, 2007). Several life strategies have developed within nematodes: from colonizers, or r-strategists, that produce many small eggs, and quickly exploit nutrient-rich habitats (e.g., Rhabditidae), through to persisters, or K-strategists, that produce few but big eggs and rarely react to transient conditions of high food availability, rather being sensitive to impacts of various origins (e.g., Dorylaimidae) (Bongers and Bongers, 1998). The colonizer-persister life strategy of nematodes can be classified in a cp scale ranging from 1 to 5. Nematodes classified as cp-1 dominate under nutrient enrichment conditions and are relatively tolerant to pollution-induced stress (e.g., Rhabditidae); the cp-2 occur under food-rich as well as food-poor conditions and are very tolerant to pollutants and other disturbances (e.g., Aphelenchidae and Cephalobidae); the cp-3 are relatively susceptible to disturbances (e.g. Diphterophoridae); the cp-4 are sensitive to pollutants (eg, Alaimidae), and finally the cp-5 are very sensitive to pollutants and other disturbances (eg, Aporcelaimidae) (Bongers and Bongers, 1998).

Nematodes are widely-used indicators of soil disturbance, quality and functioning because they are ubiquitous and abundant, and have a short response time relative to environmental impacts. Due to their morphology and feeding behaviour, nematodes are found everywhere where decomposition occurs and have key roles in regulating carbon fate and controlling soil microbial populations. Their species composition reflects substrate texture, climate, biogeography, organic inputs, and natural and anthropogenic disturbances (Bongers and Bongers, 1998; Brussaard, 1997; van den Hoogen et al., 2019; Yeates, 2003). Even though climatic variables such as temperature and precipitation have a significant impact in shaping the patterns in total soil nematode abundance, soil characteristics such as texture, soil organic carbon content and pH are by far the most important factors driving nematode abundance, suggesting that climate affects nematode density indirectly through modifying soil properties rather than directly (van den Hoogen et al., 2019). Any soil disturbance can affect soil nematode trophic structure and

total abundance (Fu et al., 2000). It has been shown that with the increasing trophic diversity of soil nematode assemblages, nutrient turnover and plant growth also increases, and that a series of nematode species can sequentially use a single resource. The basic assumption about soil conditions is that larger and more diverse nematode assemblages reflect "healthier" and are therefore "desirable" soils (Yeates, 2003). Colonizer nematodes with lower cp values (cp1 and cp2) are opportunistic nematodes indicating resource availability, on the other hand, persister nematodes with high cp values (cp4 and cp5) are indicators of system stability, food web complexity, and organisms connectance (Sanchez-Moreno and Ferris, 2018), which implies that, in order to have a more stable system with a more complex food web, the soil must have a higher abundance of higher cp value nematodes instead of lower cp value nematodes.

In the soil food web, there are two main types of energy channels, the primary production channel, and the decomposition channel. The primary production channel flows through the plant-based food chain, having photosynthetically fixed carbon as its energy source. The decomposition channel consists on the soil organic matter and its decomposition, and that is subdivided into the bacterial feeding channel, driven by bacterial decomposition of more labile nutrient-rich detritus, and the fungal channel, based on fungal decomposition of putatively more recalcitrant, nutrient-poor organic matter (Berg et al., 2001; De Ruiter et al., 1995; Wardle et al., 2003). These two different channels have linkages between them, mainly at higher trophic levels, i.e., omnivores and predators, establishing complex food web loops that have been shown to increase food web regulation and stability (Berg et al., 2001; Crotty et al., 2012; De Ruiter et al., 1995; Wall et al., 2015).

An important tool in soil studies is the application of indices that integrate information on the assemblage of nematode functional groups. The weighed mean cp value of all nematodes in a given sample, the sigma Maturity Index (MI), informs on the overall condition of the soil community, from colonization to succession following a putative disturbance. By assessing and giving different weights to the nematode guilds in soil, other indices have been proposed that evaluate different processes and components of the soil food web. The enrichment index (EI), that measures the occurrence of opportunistic bacterial and fungal feeders that respond quickly to the input of C and N sources, the structure index (SI), based on the relative abundance of nematodes in higher trophic groups and cp levels and indicates soil food web length and connectance, the channel index (CI), calculated as the proportional abundance of (cp 2) fungal feeders to the abundance of enrichment opportunist bacterial feeders, reflects the fungal or

bacterial decomposition channel in the soil, and the basal index (BI), that informs on the predominance of nematode groups that are tolerant to disturbance (cp 2). Simply plotting the EI vs. SI in faunal profiles provides a good indication of soil food web structure and enrichment, that can be explored to infer on soil quality status (Bongers and Bongers, 1998; Ferris et al., 2001; Ferris, 2010). A more recent expansion on the above indices is the calculation of metabolic footprints, that directly indicate the status of soil functions and the magnitude of ecosystem services (Sanchez-Moreno and Ferris, 2018). Metabolic footprints are based on estimates of nematode growth and respiration rates, that vary according to their lifespan, body size and activity, and reflect nutrient allocation and turnover in different food web and functional components. Metabolic footprints can be calculated by trophic group, giving an indication of e.g., belowground herbivory or predation, or by functions, such as the Enrichment and the Structure footprint (Ferris, 2010; Zhang et al., 2015). The Enrichment footprint is based on the metabolism of enrichment opportunist nematodes, such as r-strategists with lower trophic levels (cp1-2) that are most rapidly responsive to resource enrichment, being an indicator of decomposition/mineralization of nutrients. The Structure footprint takes into account metabolism of the higher trophic levels (cp3-5) that may have a regulatory function in the soil food web and which are indicative of the abundance of organisms of similar functions in non-nematode taxa, being an indicator of regulation (Ferris, 2010; Sanchez-Moreno and Ferris, 2018; Zhang et al., 2015). The integrated analysis of nematode communities is therefore a useful tool that can be applied in comparative studies to infer on belowground biodiversity and function in virtually all ecosystems.

1.3 Nature conservation and protected areas as the main global conservation strategy

Nature conservation policy is only concerned with aboveground terrestrial species and soil biodiversity has been largely ignored, resulting in a lack of information about the conservation status of most soil organisms and about the effects that nature conservation policies have on soil systems (Guerra et al., 2021). One of the main reasons for this lack of attention comes from serious technical, taxonomic and scale issues that limit the ability to establish conservation priorities for soil communities (Eisenhauer et al., 2019). Therefore, soil animals are not considered in preliminary biodiversity surveys that are undertaken for protected areas planning. At the local scale, impact studies required prior to the establishment of any type of infrastructure do not take soil taxa into account. This is also the case when it comes to determining the location and area size and shape of natural parks and reserves. This is also evident as biodiversity spatial

patterns are addressed for the identification of proprietary conservation areas at larger scales, for example biodiversity hotspots. Many impact studies particularly focus on higher plants, vertebrates and, among invertebrates, on butterflies and to a lesser extent on moths (Decaëns, 2006).

Currently, one of the main strategies for nature conservation is the establishment and maintenance of protected areas, whether on local, regional, or global scales, and a significant amount of time, effort, and resources have been invested in its prioritization and management in the last decades (Elbakidze et al., 2013; Gaston et al., 2008). The International Union for Nature Conservation (IUCN) defines protected areas as “A clearly defined geographical space, recognized, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values” (Dudley et al., 2010). To help establish a global standard for the conservation of protected sites, IUCN created a system of categorizing protected areas based on conservation objectives and management approaches (Table 1) (Dudley et al., 2010; Leberger et al., 2020; Leroux et al., 2010; Salman et al., 2020).

Table 1 - International Union for Nature Conservation (IUCN) description for protected areas management categories (adapted from IUCN - <https://www.iucn.org>).

IUCN category	Description
Strict Nature Reserve (Ia)	Protected areas that are strictly set aside to protect biodiversity and possibly geological/geomorphological features, where human visitation, use and impacts are strictly controlled and limited to ensure protection of the conservation values. Such protected areas can serve as indispensable reference areas for scientific research and monitoring.
Wilderness Area (Ib)	Protected areas that are usually large unmodified or slightly modified areas, retaining their natural character and influence, without permanent or significant human habitation, which are protected and managed to preserve their natural condition.
National Park (II)	Large natural or near-natural areas set aside to protect large-scale ecological processes, along with the complement of species and ecosystems characteristic of the area, which also provide a foundation for environmentally and culturally compatible spiritual, scientific, educational, recreational and visitor opportunities.
Natural Monument or Feature (III)	Protected areas set aside to protect a specific natural monument, which can be a landform, sea mount, submarine cavern, geological feature such as a cave or even a living feature such as an ancient grove. They are generally small areas and often have a large visitor value.
Habitat/Species Management Area (IV)	Protected areas aiming to protect a particular species or habitats and the management reflects this priority. Many will need regular, active interventions to address the requirements of particular species or to maintain habitats, but this is not a requirement of the category.
Protected Landscape/ Seascape (V)	A protected area where the interaction of people and nature over time has produced an area of distinct character with significant ecological, biological, cultural and scenic value and where safeguarding the

Table 1 - International Union for Nature Conservation (IUCN) description for protected areas management categories (adapted from IUCN - <https://www.iucn.org>) (continued).

IUCN category	Description
	Integrity of this interaction is vital to protecting and sustaining the area and its associated nature conservation and other values.
Protected area with sustainable use of natural resources (VI)	Protected areas that conserve ecosystems and habitats, together with associated cultural values and traditional natural resource management systems. They are generally large, with most of the area in a natural condition, where a proportion is under sustainable natural resource management and where low-level non-industrial use of natural resources compatible with nature conservation is seen as one of the main aims of the area.

The categories I to IV have stricter conservation measures than categories V and VI, which have more flexible land use (Dudley et al., 2010; Leberger et al., 2020; Leroux et al., 2010; Salman et al., 2020).

In parallel to the IUCN classification, Natura 2000 is the largest and the most important European Union-established network of sites chosen to ensure the long-term survival of Europe's most valuable and threatened species and habitats. The way a site is chosen is determined by what it seeks to protect. This is done in accordance with the Convention on Biological Diversity as well as the European Habitat and Birds Directives and the respective lists of threatened species. Some human activities are permitted on different levels, and management policies encourage working alongside with nature (Bartula et al., 2011; Ciobanu et al., 2019; European Commission, 2021; Jurado et al., 2019). Under the Birds Directive (79/409/EEC), according to scientific criteria, Member States designate Special Protection Areas (SPA) for 194 particularly threatened species and all migratory bird species. Member states can choose the most appropriate criteria, but must ensure that all the most suitable territories, both in terms of number and surface area, are designated. According to the Habitats Directive (92/43/EEC), the choice of sites is based on scientific criteria specified in the directive, to conserve or, where appropriate, to restore the natural habitat types and habitats of the species listed in the directive's Annexes to a favorable conservation status in their natural range. Two categories of habitats were designated: natural habitat types of Community interest (SCI) and priority natural habitat types. It should be prioritized the most threatened and/or most important conservation sites and implemented the appropriate management or restoration measures to ensure the favorable conservation status of sites (Bartula et al., 2011; Ciobanu et al., 2019; European Commission, 2021).

In Portugal, the Institute for Nature Conservation and Forests (ICNF), is the mandated Portuguese governmental body for nature and forest policies, including the management of Protected Areas and State-managed national, municipal, and communal forests of mainland Portugal. The mission of ICNF is to propose, monitor and ensure the implementation of nature and forest conservation policies, aiming at conservation, sustainable use, enhancement, enjoyment, and public recognition of the natural heritage (ICNF - Instituto da Conservação da Natureza e das Florestas, 2017). For that, the National Network of Protected Areas (RNAP) was created, with different typologies (Table 2), encompassing inland terrestrial and aquatic areas as well as marine areas in which biodiversity or other natural occurrences present, due

to their rarity, scientific, ecological, social or scenic value, a special relevance that requires specific conservation and management measures (ICNF - Instituto da Conservação da Natureza e das Florestas, 2017).

Table 2 - Institute for Nature Conservation and Forests (ICNF) description for protected areas management categories and examples from the North of Portugal (adapted from ICNF - <https://icnf.pt/>).

ICNF category	Description	Examples (North of Portugal)
National Park	Area that contains mostly representative samples of characteristic natural regions, natural and humanized landscapes, elements of biodiversity and geosites, with scientific, ecological, or educational value. It aims to protect existing natural values, preserving the integrity of ecosystems, both in terms of the constituent elements and inherent ecological processes, and the adoption of measures compatible with the objectives of their classification.	Peneda-Gerês National Park
Natural Park	Area that predominantly contains natural or semi-natural ecosystems, where the long-term preservation of biodiversity may depend on human activity, ensuring a sustainable flow of natural products and services. It aims to protect existing natural values, contributing to regional and national development, and the adoption of measures compatible with the objectives of its classification.	Montesinho Natural Park; Douro International Natural Park; Litoral Norte Natural Park; Alvão Natural Park. Tua Valley
Natural Reserve	Area that contains ecological, geological, and physiographic characteristics, or other type of attributes with scientific, ecological, or educational value, and which is not permanently or significantly inhabited.	Douro Estuary
Protected Landscape	Area that contains landscapes of harmonious interaction between human beings and nature, and that show great aesthetic, ecological or cultural value. It aims at protecting existing natural and cultural values, enhancing local identity, and adopting measures compatible with the objectives of their classification.	Serras do Porto Park Corno do Bico

Table 2 - Institute for Nature Conservation and Forests (ICNF) description for protected areas management categories and examples from the North of Portugal (adapted from ICNF - <https://icnf.pt/>) (continued).

ICNF category	Description	Examples (North of Portugal)
Natural Monument	A natural occurrence containing one or more aspects that, due to its singularity, rarity, or representativeness in ecological, aesthetic, scientific and cultural terms, require its conservation and maintenance of its integrity. It aims to protect natural values, namely notable occurrences of the geological heritage, in the integrity of their characteristics and in the immediately surrounding areas, and the adoption of measures compatible with the objectives of their classification.	

Protected areas such as national parks, nature reserves and other wilderness areas, play an important role in protecting ecosystems and species which are unlikely to survive in intensively managed areas (Branquart et al., 2008). In current times, PA also play a significant role in the mitigation and adaptation to climate change and several ecosystem services. Well-managed PA reduce rates of habitat loss, can provide carbon sequestration, and can also reduce deforestation. There is also strong evidence that PA maintain species population levels, including threatened species, better than other management approaches (Lubchenco et al., 2003; Postel & Thompson, 2005; Scharlemann et al., 2010; Soares-Filho et al., 2010; Watson et al., 2014). Ecosystem representation, functional connectivity, and management categories are relevant indicators for conservation efforts. The land-use in the matrix composition surrounding protected areas is also important to understand the role of PA for ecological sustainability, and their duration and management regimes must be understood (Elbakidze et al., 2013). However, most of the nature conservation and policy only concerns the effects of human activity and the benefits of conservation for marine environments, aboveground terrestrial species, and processes such as food production (Decaëns, 2006; Guerra et al., 2021).

1.4 Nature conservation in forest ecosystems and implications for the conservation of soils

Soil biodiversity provides a range of ecosystem services that are crucial for the well-being and integrity of above-ground biodiversity. For example, the water stored and filtered by soil will eventually flow into streams, that are crucial for the survival of aquatic species, such as salmon, often the target of protection. Soils also provide the medium for growth of terrestrial plants and provide humans with a variety of organisms and compounds used for food, medicine, and raw materials (Parker, 2010). Interactions between plants and biotic and abiotic properties of the soil play an important role in structuring plant and above-ground communities (Van der Putten et al., 2013; Van der Putten et al., 2016). Plant-soil feedbacks are shaped by three main categories of soil biota, plant enemies (microbial soil pathogens, herbivorous nematodes, insect larvae and other invertebrates), symbionts (mycorrhizal fungi, non-mycorrhizal endophytic fungi, endophytic bacteria, nitrogen-fixing microbes and plant growth-promoting microorganisms in the rhizosphere) and decomposers (organisms involved in carbon and nutrient cycles, such as nematodes, that break down litter, root exudates and soil organic matter) (Van der Putten et al., 2016). Nematodes, for example, are good indicators of decomposition and herbivory (antagonists/ enemies), playing a role in two of the three feedback groups. These categories of soil biota, influence plant growth

directly, intraspecific or conspecific, and indirectly, interspecific or heterospecific, through their influence on soil physical-chemical properties, such as pH, organic matter content, water holding capacity, temperature and soil structure. Plant–soil feedback is considered positive when it improves the performance of conspecifics and enhances the probability of that species dominating its local habitat (soilborne symbionts and decomposers generally) and on the contrary is considered negative when it makes soil less suitable for conspecifics by controlling dominance and decreasing vigour, as well as increasing the probability of that species being replaced by other species more suited to soil conditions (soilborne enemies) (Bever, 1994; Van der Putten et al., 2013; Van der Putten et al., 2016). All this will determine whether plant performance and distribution are enhanced or depressed (Bever, 1994; Van der Putten et al., 2013). Many studies have addressed the plant-soil feedbacks in forest ecosystems, for example, plant–soil interactions can maintain biodiversity and functions in tropical forest ecosystems because plants and microorganisms can modify the soil environment to adjust soil nutrient deficiency (Fujii et al., 2018), and that three coniferous plantation species affect plant-soil feedbacks and soil physical and chemical properties in semi-arid mountain ecosystems, as that they can increase soil fertility (Han et al., 2021). Plant-soil feedbacks can restore ecosystems that have been modified by anthropogenic activities since, some plant species when modifying soils may also increase their chances of establishment and growth, in areas with *Eucalyptus marginata*, *Acacia pulchella*, and *Bossiaea ornata* (Orozco-Aceves et al., 2015). Therefore, due to this strong above-belowground communities interconnection, soil biodiversity and the services it provides are an essential component of ecosystem health (Parker, 2010). It is crucial to understand how far we can impact soils without losing their functions and services. Human activities resulting in erosion (largely due to deforestation and agricultural practices involving tillage and other forms of soil disturbance), urban development, and pollution threaten soil integrity around the globe and may also have strong impacts on soil biodiversity and soil function. As a result, sites with distinct soil types that are also threatened by agriculture, urban growth, or other disturbances are likely candidates for conservation priorities (Decaëns, 2006; Parker, 2010).

If soil biota is not included in conservation actions, we will never know how they contribute to the overall picture of biodiversity in a given location, nor will we be able to pursue management actions that may improve the health and survival of other conservation targets and overall ecosystem function (Parker, 2010).

Forest areas are particularly important for conservation because of their role as a refuge and habitat for biodiversity and as ecosystem service suppliers (Cavard et al., 2011; Demissie et al., 2019; Leberger et al., 2020; Raina et al., 2011). In addition, they play an important role in the mitigation and adaptation to climate change (Elbakidze et al., 2013). However, despite playing such a significant role, little attention is given to forest-specific nature conservation. This is worrisome given the fact that forests will face particularly difficult challenges due to climate change, habitat destruction, intensified and altered land-use, and fragmentation (Milad et al., 2011). Forest ecosystems appear to be particularly vulnerable to climate change. This is due to the high anthropogenic imprint on forest composition, as well as the relatively long generation span and low migration rates of many forest ecosystem species (Jump and Penuelas, 2005; Lindner et al., 2010). Climate change is likely to change the quality of reserves by changing species distributions and composition (Skov and Svenning, 2004). The loss of a single species may have significant impacts on a protected area, particularly if the species was one of the main reasons for the reserve's designation or if the species is an essential component of a protected habitat type (Hossell et al., 2003). Climate change, in conjunction with other anthropogenic impacts, will very certainly increase the frequency, intensity, and duration of extreme events (Dale et al., 2001; Milad et al., 2011). Extreme events are predicted to have a larger impact on forest ecosystems than gradual shifts, particularly if multiple disturbances act together and thereby amplify each other or follow each other in close succession (Lindner et al., 2010). Droughts, combined with rising average temperatures, are likely to be harmful to forests, shifting species ranges, reducing forest species productivity and reproduction, and increasing mortality (Milad et al., 2011). While higher temperatures are expected to increase soil fauna activity and decomposition rates, drought may have the opposite impact or even result in the local extinction of some soil species (Dale et al., 2001; Jentsch and Beierkuhnlein, 2008).

Other influences, such as habitat destruction, intensified and altered land-use, fragmentation, and increasing population density may also be key driving forces for ecosystem change and should be minimized to increase forest ecosystem resilience (Leberger et al., 2020; Milad et al., 2011). Promoting connectivity of forest landscapes and habitat heterogeneity is likely to promote species survival, as well as changes in their composition and hence forest management, nature conservation should emphasize the idea of managing the ecosystems surrounding forest reserves (Milad et al., 2011).

1.5 Nature conservation and forest ecosystems in the North of Portugal

Forests in Northern Portugal are characterized by a remarkable diversity of ecosystems, both natural and semi-natural, as well as anthropogenic (planted forests for silviculture). The native forests are represented by common oak (*Quercus robur*) and Pyrenean oak (*Quercus pyrenaica*), best represented in the Peneda/Gerês Nature Reserve, accompanied by cork oak (*Quercus suber*). We can also find areas of native forest dominated by pine trees (maritime pine *Pinus pinaster* and Scots pine *Pinus sylvestris*). The exotic forests or anthropogenic forests are represented by gum tree plantations (*Eucalyptus globulus*), well-distributed in the Region because of their economic importance. Exotic forests can be dominated by invasive species of the genus *Acacia*, the mimosa (*Acacia delbata*), blackwood (*Acacia melanoxylon*) and golden wattle (*Acacia longifolia*) (Carmo et al., 2011; Tereso et al., 2011). Forests in Northern Portugal provide habitat for an enormous diversity of living organisms, including some of the rarest and most valuable elements of regional and national biological diversity. In comparison to the rest of the Portuguese mainland, the North of Portugal shows a high percentage of well-preserved native forest that in some cases is in expansion. Despite the importance of anthropogenic forests for silviculture, they frequently do not function as adequate refuges for biodiversity, giving greater importance to native forests, where natural processes take place with less human impact (Tereso et al., 2011).

This region is subject to a wide variety of pressures, such as invasive species and land-use change (Carmo et al., 2011; De la Fuente & Beck, 2018; Vicente et al. 2013), forest fires (Alcasena et al., 2021; Carmo et al., 2011; Marcos et al. 2019), and contamination (Bragança et al. 2019; Cachada et al., 2009). To this date, no particular attention has been given to soil biodiversity in this region but, about 25% of the area is covered by areas of conservation value, including several areas integrated in the Natura 2000 network, such as Montesinho Natural Park, Douro International Natural Park, Litoral Norte Natural Park, Alvão Natural Park, Tua Valley, Douro Estuary, Serras do Porto Park, and Corno do Bico, and the only National Park in Portugal, Peneda-Gerês National Park (ICNF - Instituto da Conservação da Natureza e das Florestas, 2017; Vicente et al. 2013).

1.6 Objectives

The overarching aim was to investigate whether and to what extent protected areas increase soil biodiversity and function in forest ecosystems. More specifically, we aimed: i) to assess differences in soil biodiversity and function between protected and non-protected areas, across a range of different forest

types, native and exotic areas, in the North of Portugal through nematode community analyses; and ii) to infer on how protected areas deal with pressures and drivers of soil biodiversity and function, integrating nematode community data with a range of other measurements and assessments taken by the SoilReCon Project - Soil Ecosystems in the XXI Century: pressures, conservation and future scenarios (PTDC/BIA-CBI/2340/2020).

2. MATERIAL AND METHODS

This work involved a wide soil sampling combined with an in-depth collection of local and remote-sensing edaphoclimatic data, as well as the biological characterization of several groups of soil organisms. A large part of this was achieved through the joint efforts of the SoilReCon project partners. Here, I present the Materials and Methods carried out for my own work, and a short summary of tasks performed by third parties (as indicated in the appropriate sections).

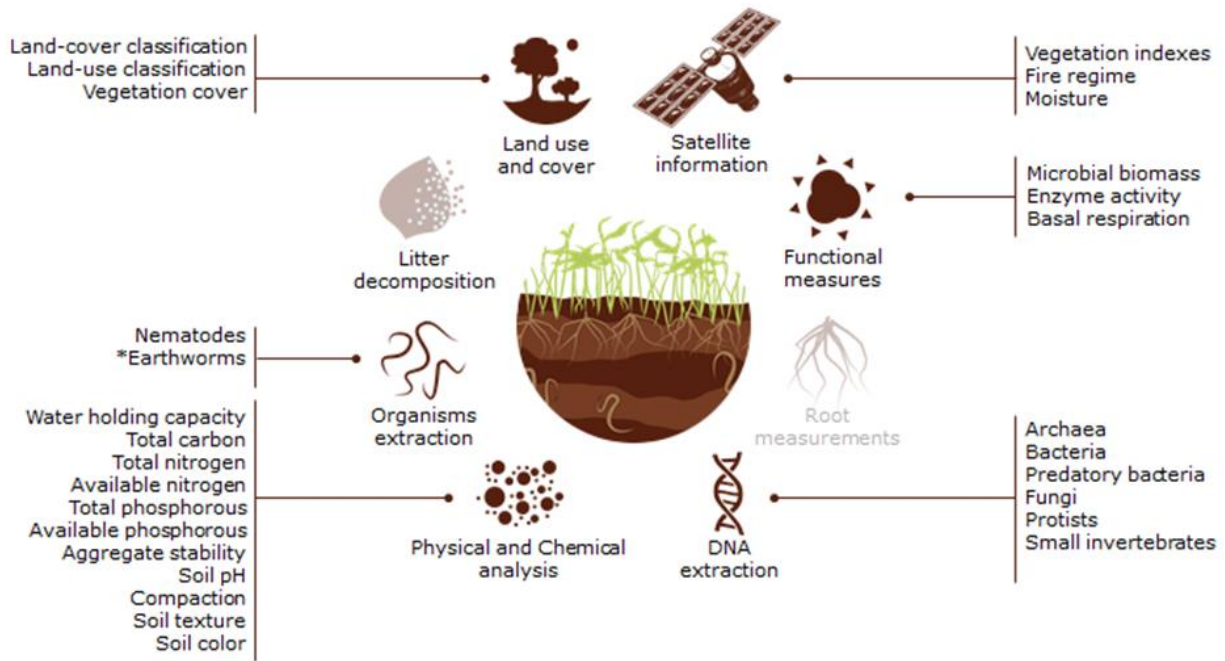


Figure 1 - Environmental and edaphoclimatic data collected for each sampling point by the SoilReCon project team (PTDC/BIA-CBI/2340/2020).

2.1 Sampling and data collection

In order to have an adequate representation of the local environmental and social-economic conditions in the North of Portugal, the SoilRecon project team implemented a random sampling scheme considering a 20x20km grid across the entire region. For each grid cell, six sampling sites were randomly selected, one per major land-use type, i.e., urban areas, pastures, agricultural areas (subdivided into permanent and annual crops), and forests (subdivided into those dominated by native or exotic trees). Due to access restrictions and projected land use error, a total of 406 out of 420 potential sites were sampled. This sampling design allowed the homogeneous survey of the entire region and, through the stratification

according to land-use type, it was guaranteed that different habitats, pressures, and drivers were well represented within the regional environmental gradient. The samples were taken in autumn, from October 2021 to December 2021. For the purposes of this work, only forest samples (n=127) were considered, containing native or exotic tree species (exotic=57, native=70; Figure 1), both inside and outside of the protected areas (protected=38, non-protected=89), such as National Parks (n=7 samples), Natural Parks (n=17 samples), Protected Landscapes (n=1 sample), and Sites of Community Interest (n=13 samples). The protected areas covered were the protected landscape of Valongo (n=1 sample), the Peneda-Gerês National Park (n=7 samples), the Montesinho/Nogueira Natural Park (n=9 samples), Douro International Natural Park (n=2 samples), and Alvão Natural Park (n=4 samples). Sites of community interest included the Mines of St. Adrian (n=1 sample), the Serras da Freita and Arada (n=1 sample), Montemuro (n=2 samples), Serra d'Arga (n=1 sample), Corno do Bico (n=1 sample), Sabor and Maris rivers (n=2 samples), and Romeu (n=1 sample) (Figure 2).

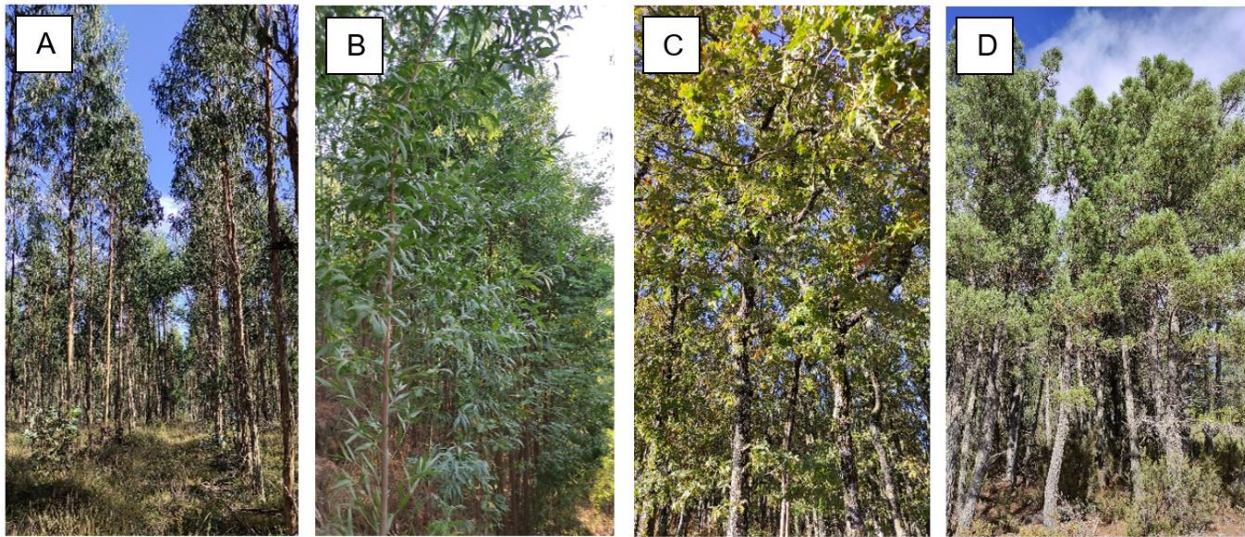


Figure 2 – Examples of the general appearance of the areas dominated by exotic tree species (A) *Eucalyptus globulus* and (B) *Acacia melanoxylon*; and native tree species (C) *Quercus robur* and (D) *Pinus pinaster*.

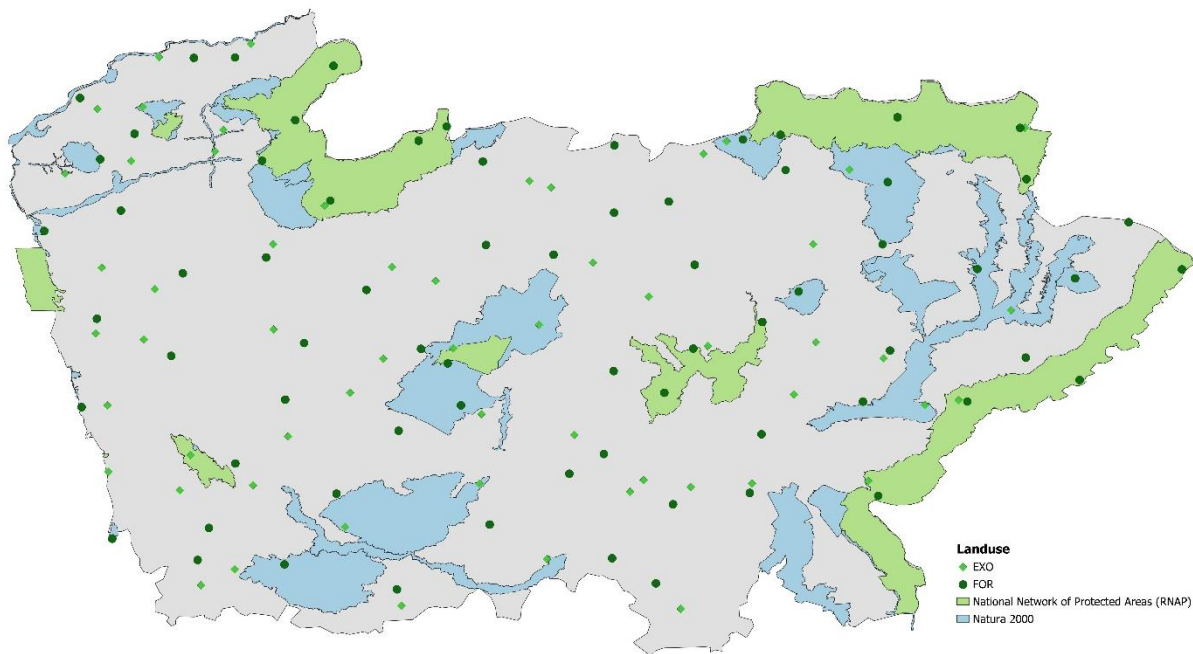


Figure 3 - Soil sampling locations with the protected areas limits in the North of Portugal. The points represented in dark green correspond to native forest areas (FOR), whereas the points represented in light green correspond to exotic forest areas (EXO). Areas represented in blue correspond to areas integrated in Natura 2000, whereas the areas represented as green, correspond to areas integrated in the National Network of Protected Areas (RNAP).

A composite soil sample was obtained from each site, composed of 9 subsamples collected at 15 m intervals and the central point of a virtual 30 x 30 m square, each taken to a depth of 10 cm, the most biologically active zone (van den Hoogen et al. 2019). In each of the 9 subsamples, the loose litter layer was removed prior to sampling. The sub-samples were thoroughly and carefully mixed and any large plant material (roots, leaves, twigs, moss, grasses, etc.), big rocks and big animals (larger than a thumbnail) were removed. From the composite sample, a 100 ml- sample was kept for nematode analysis. Further samples were also taken for functional analysis, determination of the physical and chemical properties of the soil, and for molecular analysis of several taxa. Soil bulk density was determined in an undisturbed soil cylinder collected next to the central point of the sampling site. All samples were labeled with barcodes for

identification and refrigerated until processed. For nematode analysis, soils were processed within a maximum of two months after collection.

For each sampling point a set of environmental and edaphoclimatic data was collected by the SoilReCon project team, such as, for example, vegetation cover, land-use classification, soil pH, soil organic carbon, among others. In this characterization, information was also gathered from several other datasets, such as climatic and elevation datasets.

2.2 Nematode extraction and identification

Nematodes were extracted from 100 ml of soil from each sample using the tray method, an adaptation of the Baermann Funnel (Whitehead & Hemming 1965) (Figure 4). First, the stones and bulky debris present in the soil samples were discarded, followed by the homogeneous distribution of the 100 ml of this soil in a plastic tray with a net and a sheet of paper tissue. Afterwards, the edges of the tissue were folded, and the soil was then flooded with water. The trays were placed in a room without agitation and disturbances, allowing nematodes to move through the tissue and into the tray and remain suspended in water, in a process that took approximately 72 hours. It is relevant to note that forest soils are more hydrophobic, so it was necessary to wet the soil of the trays with the help of a squirt bottle. Finally, after the 72 hours, the net with the soil was carefully removed and the nematode suspension retained in the tray was concentrated. For this, the suspension was poured through a 20 μm sieve and nematodes collected in a smaller volume of 10-20 ml.

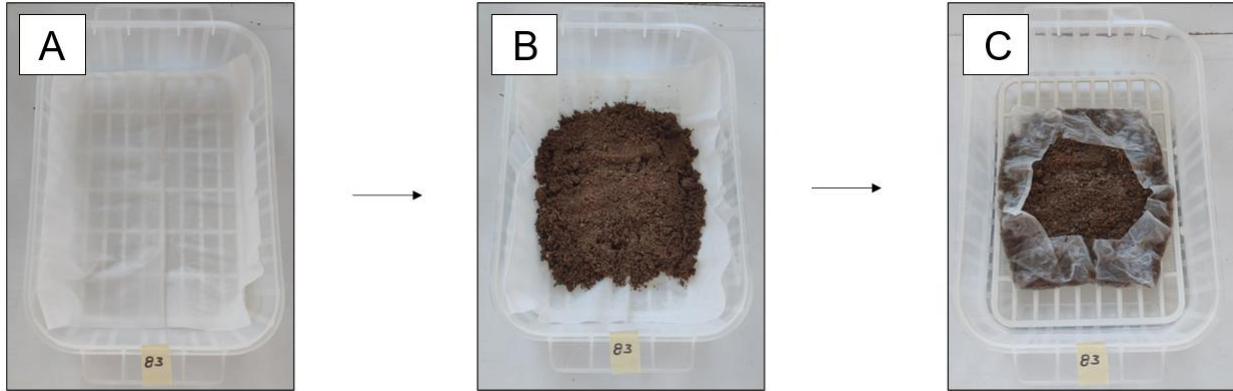


Figure 4 - Illustration of the tray method for nematode extraction from soil. (A) Plastic tray with a net and a sheet of paper tissue; (B) 100 ml of soil homogeneously distributed in a tray; (C) Tray and soil flooded with water.

Using an inverted microscope (Leica DMi1), the first 100-200 observed nematodes were classified in trophic groups through the observation of mouth and upper digestive tract (Yeates et al., 1993), to family or up to genus level to be classified in functional groups (Bongers, 1990). For the identification of nematodes, simplified keys were used (Ferris 2011; Goodey, 1963; Mai and Mullin, 1996; Tarjan et al., 1977). To estimate total numbers of nematodes in suspensions, they were quantified in nematode-counting plates (obtained from Wageningen University, the Netherlands).

2.3 Statistical analysis

All nematode collected data was analysed using the NINJA software: Nematode Indicator Joint Analysis (Sieriebriennikov et al. 2014) to calculate indices (Ferris et al., 2001) and metabolic footprints (Ferris 2010). The calculation of the indices, enrichment index and structure index, and metabolic footprints: enrichment, structure, bacterivore, fungivore, herbivore, omnivore, and predator (Table 3), allowed to plot faunal profiles and radar charts of nematode metabolic footprints (Sanchez-Moreno & Ferris, 2018). In the faunal profiles, the enrichment and structure components of the metabolic footprint were represented at the intersection of the indices (enrichment and structure indices). However, since the units of the indices are in percentage and the footprints are in carbon utilization per unit volume or weight of soil, the footprints can be divided by an adjustable scalar, k , in this case 16, that was maintained constant for all footprints on the graphical representation, for acceptable visual comparison of footprints of different types and locations. The x-axis coordinates were hence calculated as $SI-0.5*S_FP/k$ and $SI+0.5*S_FP/k$ and the

y-axis coordinates as $EI - 0.5 \cdot E_{FP}/k$ and $EI + 0.5 \cdot E_{FP}/k$. The functional metabolic footprint is represented by sequentially connecting the points: $SI - 0.5 \cdot S_{FP}/k$, EI ; SI , $EI + 0.5 \cdot E_{FP}/k$; $SI + 0.5 \cdot S_{FP}/k$, EI ; SI , $EI - 0.5 \cdot E_{FP}/k$; and $SI - 0.5 \cdot S_{FP}/k$, EI (Ferris, 2010).

Table 3 - Description and calculation of formulas of enrichment and structure indices and enrichment, structure, bacterivore, fungivore, herbivore, omnivore, and predator metabolic footprints. In the indices, e is the enrichment component, s is the structure component, and b is the basal component. As for the footprints, n is the numerical abundance and FP the median footprint of each nematode trophic group in each cp category (adapted from Ferris et al., 2001 and Ferris 2010).

Index/Footprint	Description	Formula	Reference
Enrichment index	Measures the occurrence of opportunistic bacterial and fungal feeders that respond quickly to the input of C and N sources.	$EI = 100 \cdot e / (e + b)$	Ferris et al., 2001
Structure index	Based on the relative abundance of nematodes in higher trophic groups and cp levels and indicates soil food web length and connectance.	$SI = 100 \cdot s / (s + b)$	Ferris et al., 2001
Enrichment footprint	Metabolic footprint of the enrichment opportunist nematodes that are most rapidly responsive to resource enrichment, being an indicator of decomposition/mineralization of nutrients.	$EFP = \sum 1 \cdot FP1$	Ferris 2010
Structure footprint	Metabolic footprint of the higher trophic levels that may have a regulatory function in the soil food web and which are indicative of the abundance of organisms of similar functions in non-nematode taxa, being an indicator of regulation.	$SFP = (\sum 3 \cdot FP3 + \sum 3 \cdot FP3 + \sum 3 \cdot FP3)$	Ferris 2010
Bacterivore footprint	Metabolic footprint of bacterial-feeding nematodes, indicator of carbon and energy flow through the bacterial decomposition channel.	$BFP = (\sum cp - 1n \cdot FP1 + \sum cp - 2n \cdot FP2 + \sum cp - 3n \cdot FP3 + \sum cp - 4n \cdot FP4 + \sum cp - 5n \cdot FP5)$	Ferris 2010
Fungivore footprint	Metabolic footprint of fungal-feeding nematodes, indicator of carbon and energy flow through the fungal decomposition channel.	$FFP = (\sum cp - 1n \cdot FP1 + \sum cp - 2n \cdot FP2 + \sum cp - 3n \cdot FP3 + \sum cp - 4n \cdot FP4 + \sum cp - 5n \cdot FP5)$	Ferris 2010

Table 3 - Description and calculation of formulas of enrichment and structure indices and enrichment, structure, bacterivore, fungivore, herbivore, omnivore, and predator metabolic footprints. In the indices, e is the enrichment component, s is the structure component, and b is the basal component. As for the footprints, n is the numerical abundance and FP the median footprint of each nematode trophic group in each cp category (adapted from Ferris et al., 2001 and Ferris 2010) (continued).

Index/Footprint	Description	Formula	Reference
Herbivore footprint	Metabolic footprint of herbivorous nematodes, indicator of carbon and energy flow through herbivory (primary production channel).	$HFP = (\sum_{cp} - 1n*FP1 + \sum_{cp} - 2n*FP2 + \sum_{cp} - 3n*FP3 + \sum_{cp} - 4n*FP4 + \sum_{cp} - 5n*FP5)$	Ferris 2010
Omnivore footprint	Metabolic footprint of omnivorous nematodes, indicator of regulation.	$OFP = (\sum_{cp} - 1n*FP1 + \sum_{cp} - 2n*FP2 + \sum_{cp} - 3n*FP3 + \sum_{cp} - 4n*FP4 + \sum_{cp} - 5n*FP5)$	Ferris 2010
Predator footprint	Metabolic footprint of predatory nematodes, indicator of regulation.	$PFP = (\sum_{cp} - 1n*FP1 + \sum_{cp} - 2n*FP2 + \sum_{cp} - 3n*FP3 + \sum_{cp} - 4n*FP4 + \sum_{cp} - 5n*FP5)$	Ferris 2010

The analyses done to test the effects of land use and protection on soil ecosystem functions were performed using RSTUDIO software (version 2022.07.1+554, RStudio Team 2022). Pairs of sites including native and exotic or protected and non-protected forests areas were done using the distance in meters between sites, calculated through the site coordinates using the package “geosphere” (Karney, 2013). Resulting data and environmental variables were transformed using “scale”, for environmental variables, and “log”, for the functional variables, to normalize the data. Then all parameters were analysed and compared between forest types (exotic and native forest areas) and between protected and non-protected areas using t-test-type statistics or their combinations by two-way repeated measures ANOVA. Soil ecosystem functions were considered for all pairs to significantly differ if the p-value from t-test, parametric test, or two-way repeated measures ANOVA was lower than 0.05. The influence of the environment and edaphoclimatic variables on soil ecosystem functions were determined by Canonical Correspondence Analysis (CCA) using the PAST software (Hammer et al., 2001). First, to reduce the number of variables, in RSTUDIO, the Variance Inflation Factor (VIF), function vif() from the package “car” (Fox and Weisberg, 2019), was used. Variables were highly correlated if VIF value >5 so, variables with values above 5 were eliminated one by one, thus obtaining a shortlist of 24 variables. This list was further shortened through the analysis of Spearman correlations between variable pairs. Among these 24 variables, the ones who were significantly correlated ($p < 0.05$) and with a correlation coefficient approximately larger than 0.5 or smaller than -0.5 (Annex I), were omitted, thus obtaining a final shortlist of 15 environmental and edaphoclimatic variables, that were used in the CCA. The CCA was performed to ascertain the influence of the environmental and edaphoclimatic variables with a range of different functional variables, enrichment index, structure index, alpha diversity, and metabolic footprints, and, separately, nematode trophic groups, or nematode families/genera. Variables were transformed using “scale”, for environmental variables, and “log”, for the functional variables, to normalize the data, as described above. First, through permutation analysis, it was seen if the CCA axes were significant ($p < 0.05$), then, taking into account the correlation values of each environmental factor/variables, their contribution to the dispersion of values related to the different functional variables and nematode community was assessed.

3. RESULTS

3.1 Effect of Land use and Protection in nematode abundance and diversity

Soils in native forests had a greater abundance of free-living nematodes (bacterivores, fungivores, omnivores and predators) than in the exotic forests ($p < 0.05$). This was not observed for plant-parasitic nematodes, whose abundance did not differ statistically between the two types of land use (Figure 5A), nor according to forest protection status (protected vs. non-protected areas) (Figure 5B). These nematodes seemed more abundant in non-protected forests than in protected forests; the latter also tending to have a greater abundance of bacterial-feeding, omnivorous, and predatory nematodes ($p > 0.05$), with predator nematodes being significantly greater in protected areas. Native forests in non-protected areas tended to have a greater abundance of bacterial-feeding, omnivorous and predatory nematodes, that were scarcer in exotic forests ($p > 0.05$) (Figure 7). In native forests in protected areas, fungal-feeding and herbivorous nematodes were generally more abundant, in contrast with the smaller numbers detected in the exotic forests both in protected and non-protected areas ($p > 0.05$). Contrary to abundance, nematode alpha diversity did not significantly differ between the two types of land use (Figure 6A) but was significantly greater in soils from protected areas (Figure 6B). Nematode alpha diversity was higher in native forests in protected areas and lower in exotic forests both in protected and non-protected areas ($p < 0.05$) (Figure 7F).

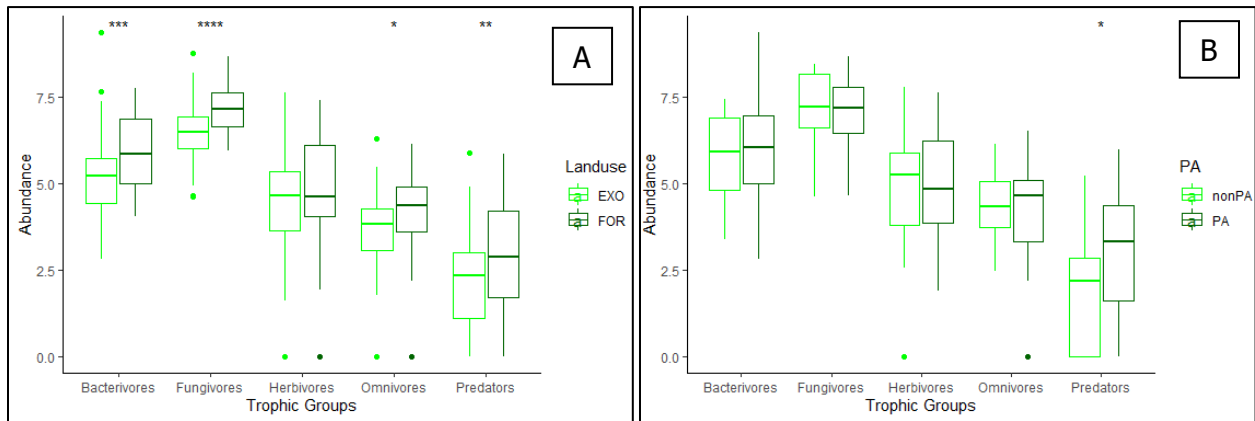


Figure 5 - Box plot representing the abundances of the nematodes trophic groups in forest areas (A) containing native (FOR) or exotic (EXO) tree species and (B) inside (PA) or outside (nonPA) protected areas. *: significant difference ($p < 0.05$); **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

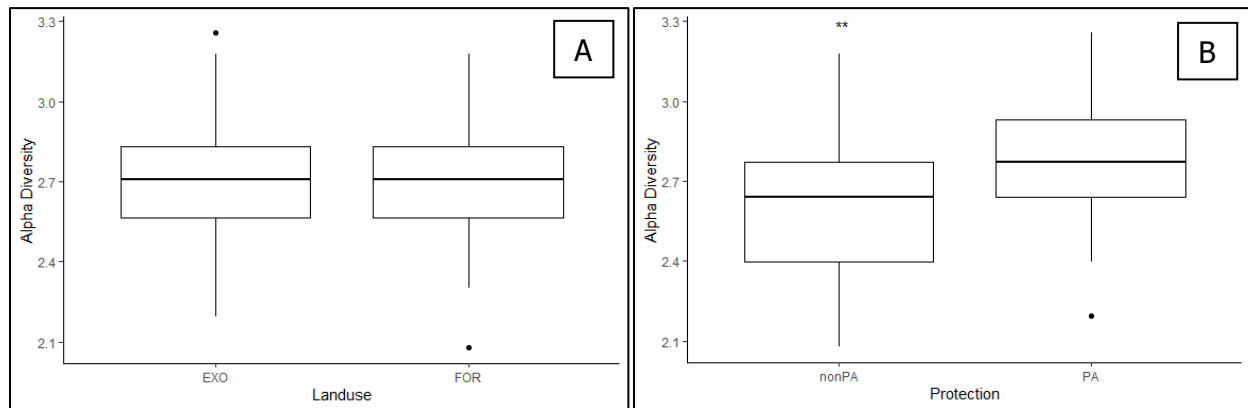


Figure 6 - Box plot representing the nematodes alpha diversity in forest areas (A) containing native (FOR) or exotic (EXO) tree species and (B) inside (PA) or outside (nonPA) protected areas. *: significant difference ($p < 0.05$); **: $p \leq 0.01$.

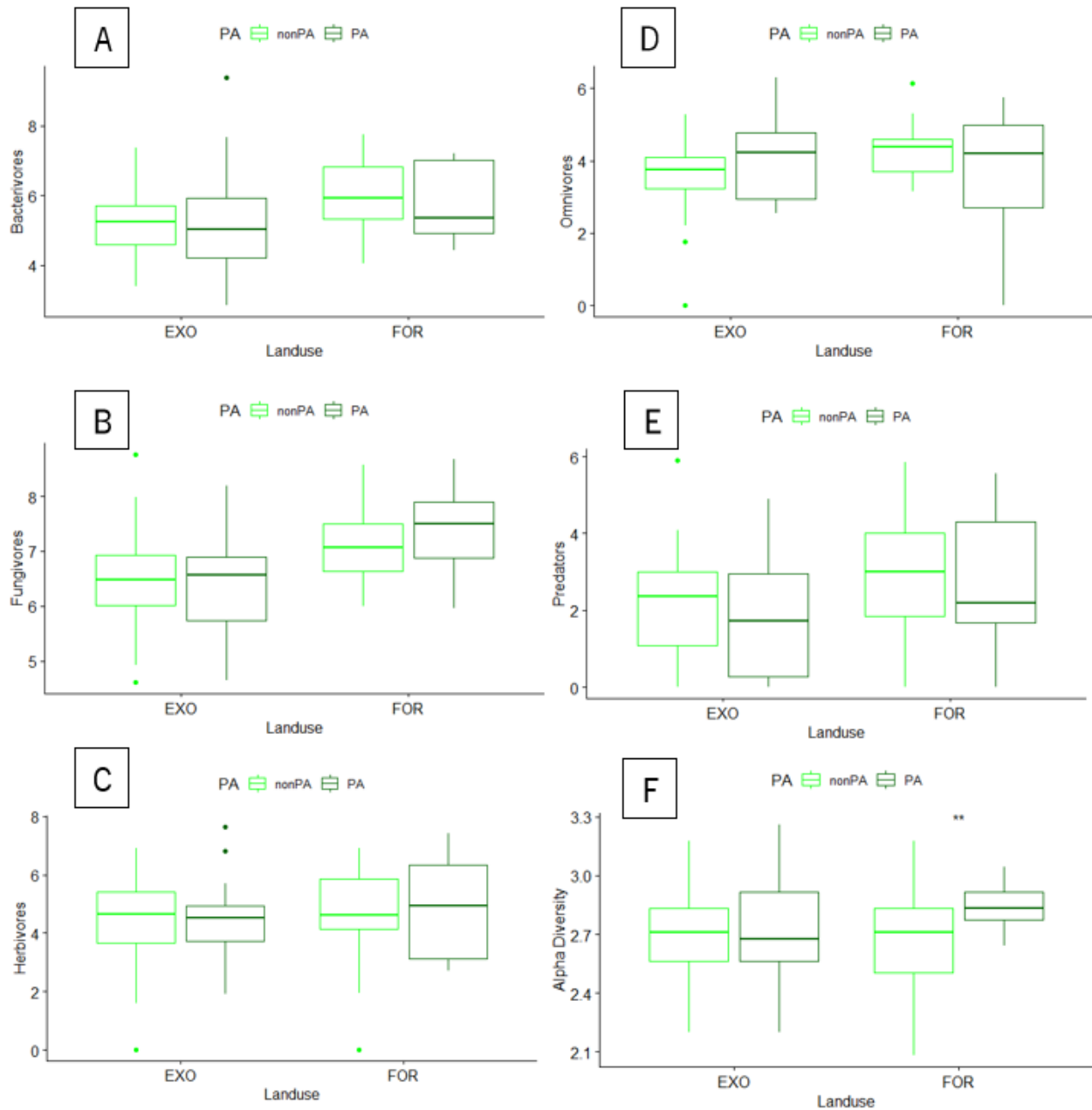


Figure 7 - Box plot representing the abundances of (A) bacterivorous nematodes; (B) fungivorous nematodes; (C) herbivorous nematodes; (D) omnivorous nematodes; (E) predator nematodes; and (D) nematodes alpha diversity, in different forest types inside or outside protected areas. *: significant difference ($p < 0.05$); **: $p \leq 0.01$.

3.2 Effect of Land use and Protection in Soil Functions

Native forests soils had significantly larger Enrichment and Structure footprints than exotic forests, indicative of a higher rate of nutrient mineralization and availability, and greater level of natural regulation, respectively. However, having a Structure footprint larger than the Enrichment footprint, soil communities in native forests did not have a great availability of nutrients, there was a greater investment in food web complexity and food chain length. On the other hand, the representation of metabolic footprints of exotic forests had a rhomboid shape closest to a square, that means that the productivity and turnover rates of the enrichment indicators, representative of the prey, are sufficient to maintain the needs of the predators, the structure indicators, so the system was in metabolic balance (Ferris, 2010) (Figure 8C). Protected areas tended to have a greater Enrichment and Structure footprints than non-protected areas, investing in food web complexity and food chain length ($p>0.05$). Enrichment and Structure indices were generally higher for native forests, as well as for protected areas, in contrast to exotic forests and non-protected areas, suggesting that native and protected forests had a bigger occurrence of opportunistic bacterial and fungal feeders and a higher relative abundance of nematodes in higher trophic groups and cp levels, but these effects were not significant (Figure 8B).

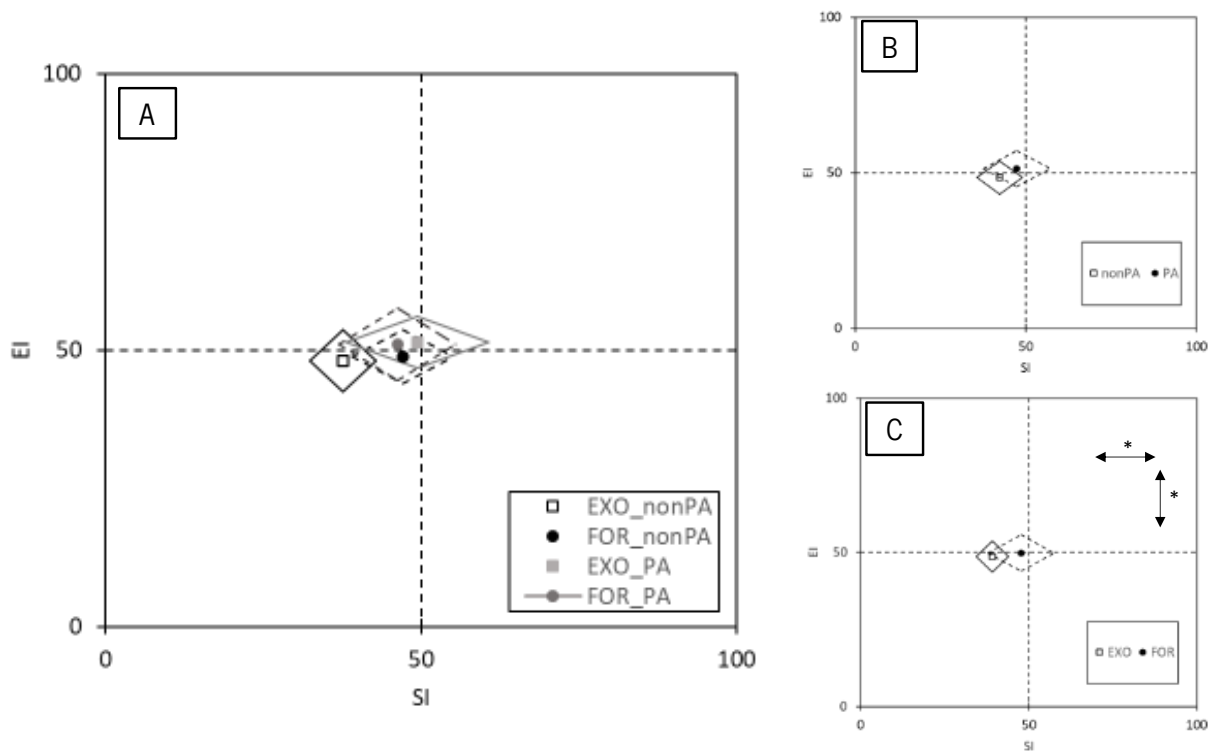


Figure 8 - Faunal profile representing the structure and enrichment conditions of the soil food web for (A) combinations of land use and protection; (B) forest areas with native (FOR) or exotic (EXO) tree species; (C) forest areas inside (PA) or outside (nonPA) protected areas. *: significant difference ($p < 0.05$).

Nutrient allocation in soil food webs both in native and in exotic forests was larger in the decomposer food web (larger metabolic footprints of bacterivores, fungivores and omnivores) than in the primary production channel (smaller herbivory footprint) ($p < 0.05$). In addition, native forests had significantly greater overall nematode metabolic footprints than exotic forests, except for the herbivore footprint, for which significant differences were not detected. The nutrient mineralization, assessed by the metabolic footprints of bacterivores and fungivores, was significantly greater in forest areas dominated by native tree species than in those dominated by exotic tree species. The suppressive service suggested by the omnivore footprint was significantly greater in native forest areas than in exotic forest areas (Figure 9B). Irrespective of protection status, forests seemed to have larger bacterivore, fungivore and omnivore footprints than herbivore footprints ($p > 0.05$), again suggesting a greater allocation of nutrients to the decomposition process rather than herbivory pressure. The predator footprint showed a significant difference between the two types of protection ($p < 0.05$), with forest soil in protected areas having a larger

predator footprint (Figure 9C), while nutrient mineralization and suppressive service tended to be slightly greater (non-significant; $p>0.05$) in protected areas than in non-protected areas (Figure 9C).



Figure 9 - Nematode metabolic footprints as indicators of the magnitude of the ecosystem services performed by each nematode functional group for (A) combinations of land use and protection; (B) forest areas with native (FOR) or exotic (EXO) tree species; (C) forest areas inside (PA) or outside (nonPA) protected areas. *: significant difference ($p < 0.05$).

3.3 Influence of the environmental and edaphoclimatic variables on soil biodiversity and functions

The influence of the environmental and edaphoclimatic variables on soil ecosystem functions were analysed and expressed in a CCA biplot. Environmental variables explained 15% of the variance in nematode community indices and alpha diversity, and the percentage of variance explained was 12% for axis 1 and 3% for axis 2, neither of which was significant. Still, considering the correlations to the axes, soil

organic carbon, moisture, and deciduous forest cover were the variables that most contributed to the dispersion of values related to the structure index (SI), while for the enrichment index (EI), it was P and apparent density. The dispersion of values related to the alpha diversity of the nematodes does not appear to have been explained by any of the variables (Figure 10A). In the analysis of the effects of environmental variables on nematode metabolic footprints, variables explained 16% of the variance, and the percentage of variance explained was 9% for axis 1 and 3% for axis 2, but neither of them was significant. Soil organic carbon and moisture were the variables that most contributed to the dispersion of values related to the predator footprint (PR_FP), whereas for the herbivore footprint (H_FP), P was the variable that most contributed to the dispersion. As for the omnivore, fungivore, bacterivore, structure, and enrichment footprints, their dispersion was associated with apparent density, albedo, distance to rivers, mean annual temperature, distance to urbanization and pH (Figure 11A). No apparent clear distinction in data distribution according to land use (Figure 10B; Figure 11B), protection status (Figure 10C; Figure 11C), or the interconnection of the two factors (Figure 10D; Figure 11D), was found, as 95% ellipsoids for datapoints in each group mostly overlapped with the others.

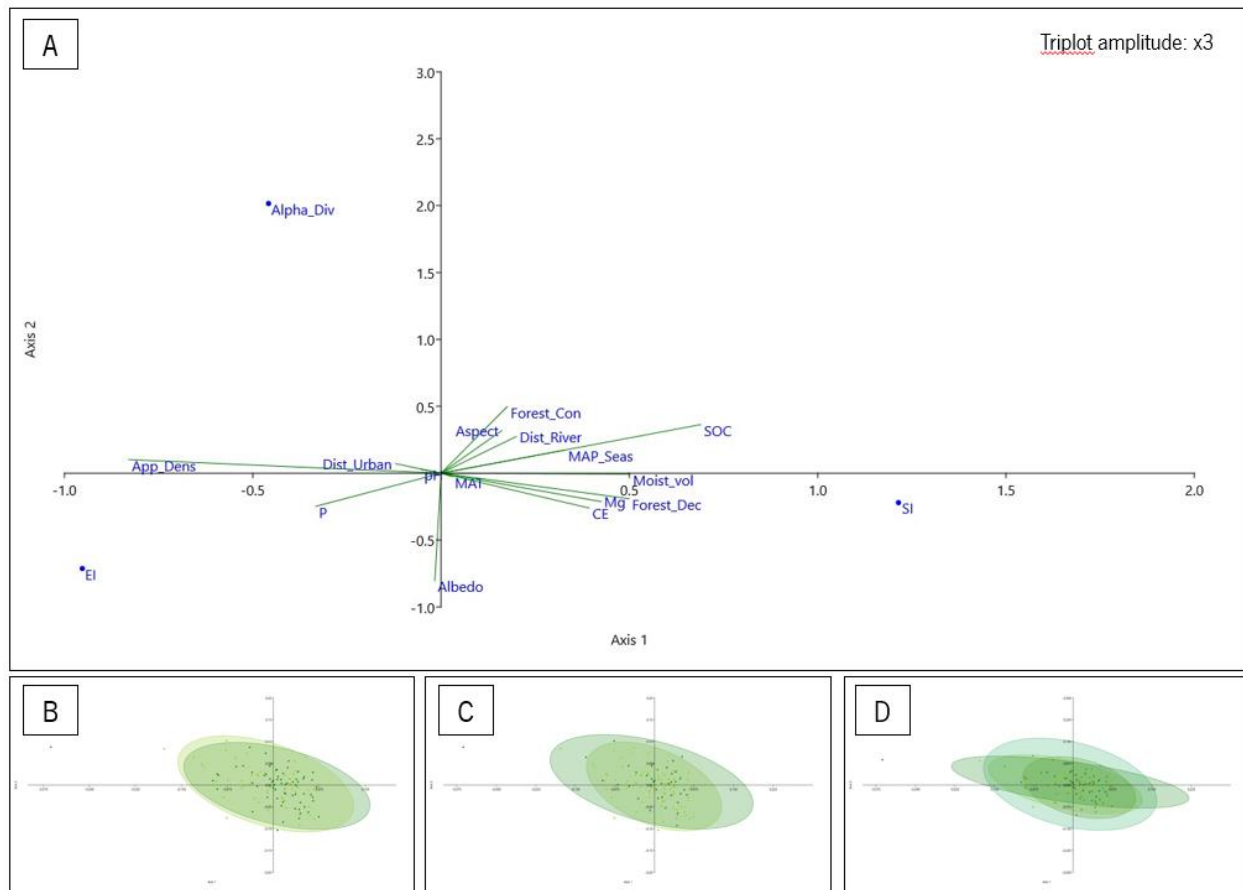


Figure 10 - Canonical correspondence analysis of the influence of the environmental and edaphoclimatic variables on soil ecosystem functions. Eigenvalues were 0.0009998 and 0.0002407 for the first and the second axis, respectively. A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and the Alpha Diversity, Structure and Enrichment Index (Triplot amplitude: x3). (B) overlapping of 95% ellipses for the land use groups. (C) overlapping of the 95% ellipses for the protection groups. (D) overlapping of the 95% ellipses for the group combinations of land use plus protection groups. Alpha_Div: Alpha Diversity; SI: Structure Index; EI: Enrichment Index; Forest_Dec: Deciduous Forest Cover; SOC: Soil Organic Carbon; Forest_Con: Coniferous Forest Cover; MAP_Seas: Mean Annual Precipitation Seasonal; CE: Electric Conductivity; Moist_vol: Moist by volume of soil; Mg: Magnesium; P: Phosphorous; Dist_Urban: Distance to Urbanization; App_Dens: Apparent Density; Dist_River: Distance to Rivers; MAT: Mean Annual Temperature.

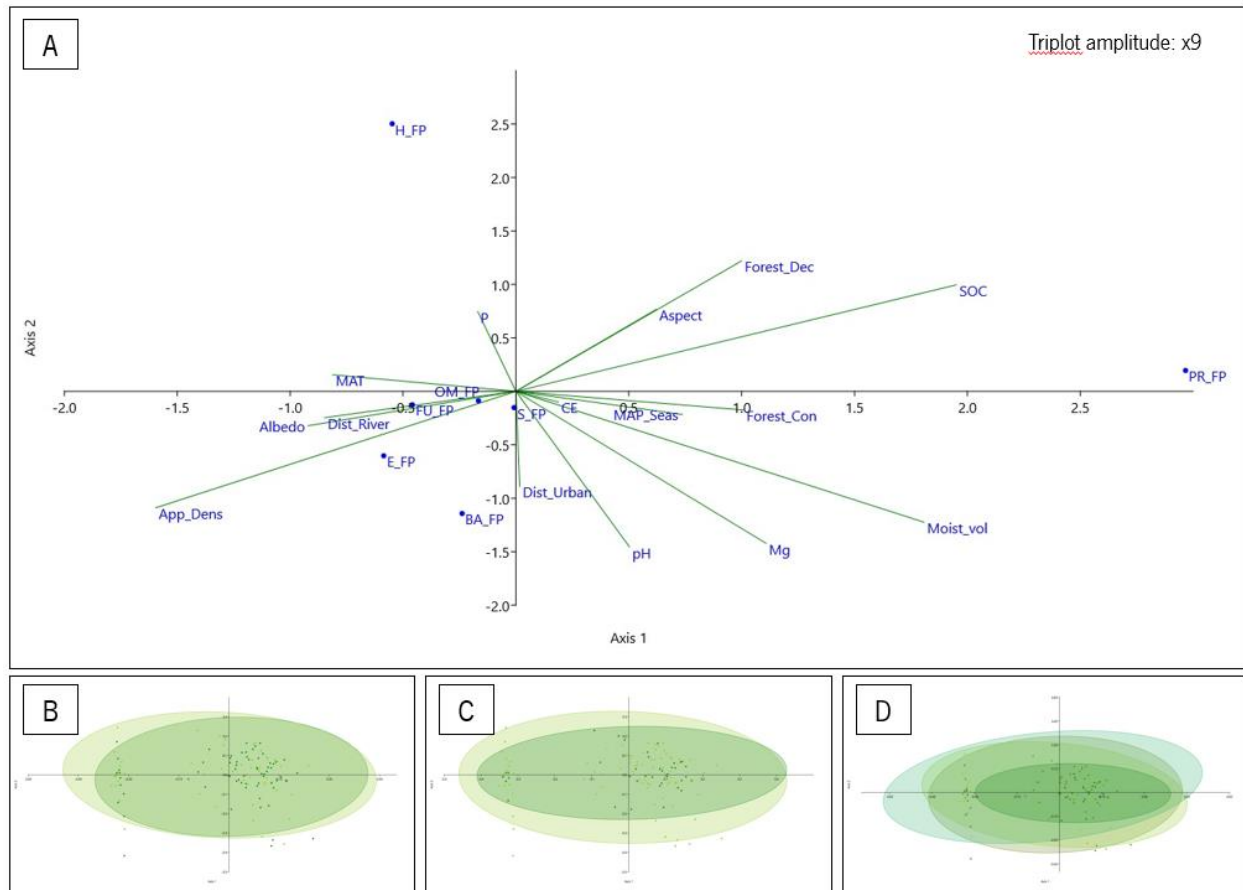


Figure 11 - Canonical correspondence analysis bi-plots of the influence of the environmental and edaphoclimatic variables on soil ecosystem functions. Environmental variables are marked by arrows. Eigenvalues were 0.004099 and 0.001445 for the first and the second axis, respectively. (A) Canonical correspondence analysis biplot representing the environmental variable vectors and the Enrichment, Structure, Bacterivore, Fungivore, Herbivore, Omnivore, and Predator Footprints (Triplot amplitude: x9). (B) overlapping of 95% ellipses for the land use groups. (C) overlapping of the 95% ellipses for the protection groups. (D) overlapping of the 95% ellipses for the group combinations of land use plus protection groups. E_FP: Enrichment Footprint; S_FP: Structure Footprint; BA_FP: Bacterivore Footprint; FU_FP: Fungivore Footprint; H_FP: Herbivore Footprint; OM_FP: Omnivore Footprint; PR_FP: Predator Footprint; Forest_Dec: Deciduous Forest Cover; SOC: Soil Organic Carbon; Forest_Con: Coniferous Forest Cover; MAP_Seas: Mean Annual Precipitation Seasonal; CE: Electric Conductivity; Moist_vol: Moist by volume of soil; Mg: Magnesium; P: Phosphorous; Dist_Urban: Distance to Urbanization; App_Dens: Apparent Density; Dist_River: Distance to Rivers; MAT: Mean Annual Temperature.

3.4 Influence of the environmental and edaphoclimatic variables on nematode trophic groups

For the nematode trophic groups, the environmental variables considered explained 17% of the variance, and the percentage of variance explained was 12% for axis 1 and 3% for axis 2, being significant for axis 1 and non-significant for axis 2. Considering correlations to the axes, apparent density, distance to rivers, and mean annual temperature were the variables that most contributed to the dispersion of values related to the abundances of fungivores and bacterivores, while for omnivores it was moisture, and coniferous forest cover. The abundance of predators was associated with soil organic carbon, deciduous forest cover and moisture, finally, albedo and P, were the variables that most contributed to the dispersion of values related to herbivores (Figure 12A). Considering axis 1, that is significant, it was possible to see that predator nematodes were clearly separated from the other trophic groups; both predators and omnivores, nematodes closely linked to complex and long food chains, were on the positive side of the axis, being positively associated with soil organic carbon and moisture and negatively associated with apparent density. The bacterivores, fungivores and herbivores were on the negative side of the axis, being negatively correlated with soil organic carbon and moisture and positively associated with apparent density. Furthermore, bacterivores were closer to the origin, being less affected by the environmental variables, meaning that they were basically in any type of environment. Herbivores were more separated from the other trophic groups considering the axis 2, associated with the forest deciduous cover, but the axis was non-significant. A clear distinction in data distribution according to land use (Figure 12B), protection status (Figure 12C), or the interconnection of the two factors (Figure 12D), was not apparent, as 95% ellipsoids for datapoints in each group mostly overlapped with the others.

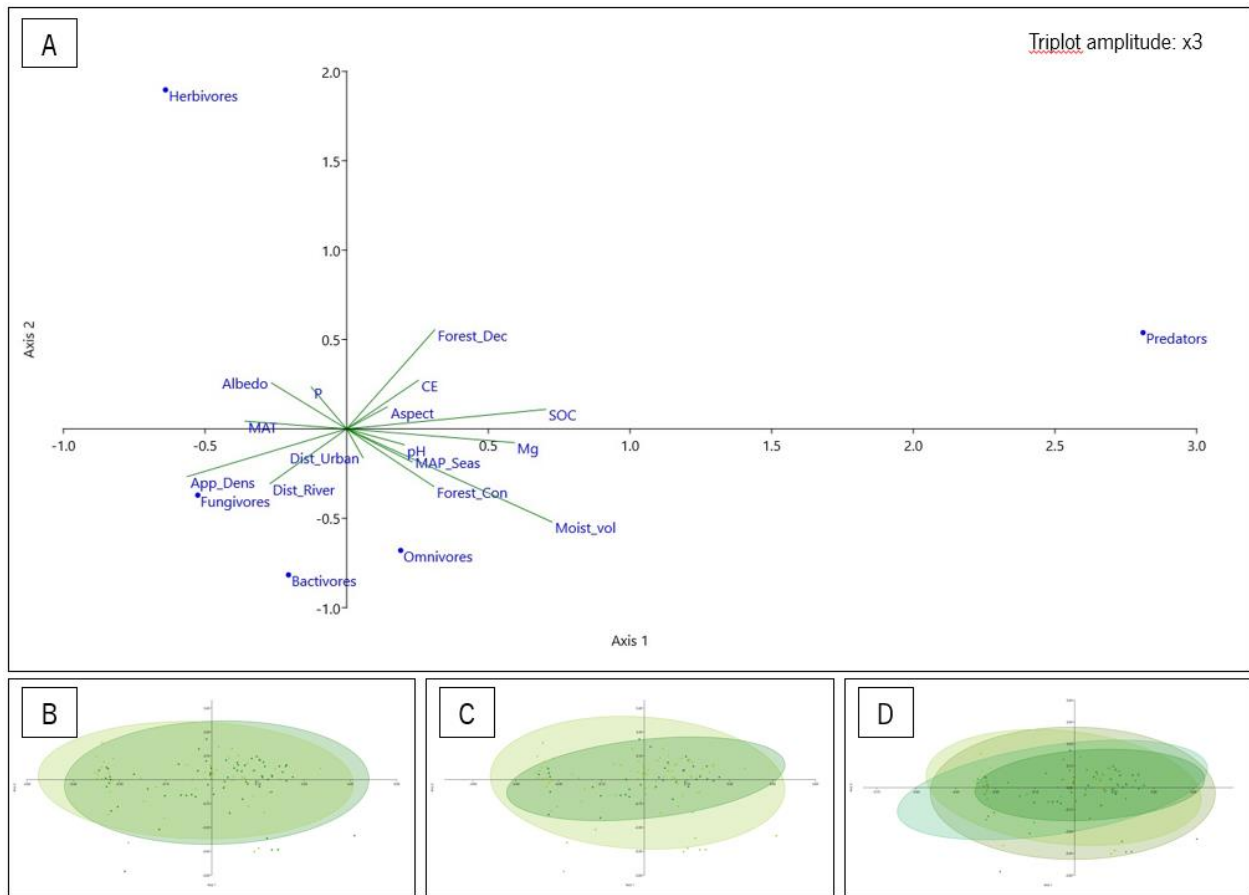


Figure 12 - Canonical correspondence analysis bi-plots of the influence of the environmental and edaphoclimatic variables on nematode trophic groups. Environmental variables are marked by arrows. Eigenvalues were 0.008704 and 0.002156 for the first and the second axis, respectively. (A) Canonical correspondence analysis biplot representing the environmental variable vectors and the bacterivorous, fungivorous, herbivorous, omnivorous and predator nematodes (Triplot amplitude: x3). (B) overlapping of 95% ellipses for the land use groups. (C) overlapping of the 95% ellipses for the protection groups. (D) overlapping of the 95% ellipses for the group combinations of land use plus protection groups. Forest_Dec: Deciduous Forest Cover; SOC: Soil Organic Carbon; Forest_Con: Coniferous Forest Cover; MAP_Seas: Mean Annual Precipitation Seasonal; CE: Electric Conductivity; Moist_vol: Moist by volume of soil; Mg: Magnesium; P: Phosphorous; Dist_Urban: Distance to Urbanization; App_Dens: Apparent Density; Dist_River: Distance to Rivers; MAT: Mean Annual Temperature.

3.5 Influence of the environmental and edaphoclimatic variables on taxonomical diversity

Taking into account nematode taxonomical diversity, variables explained 15% of the variance, and the percentage of variance explained was 3% for axis 1 and 2% for axis 2, neither of them significant. It was possible to see that in this CCA there was no clear patterns on the distribution of nematode taxonomical diversity considering the environmental and edaphoclimatic variables. The large triplot amplitude of x9 chosen for visual representation in Figure 13A reflects the small environmental variable vector sizes, indicative of their small influence on the abundance of nematode taxa. There seemed to be no pattern for the bacterivore nematode taxa, however fungivores were generally on the right side of the graph, axis 1 positive, associated with forest deciduous cover, soil organic carbon and electric conductivity. The same was seen for the predators. The distribution of Dorylamidae seemed to be influenced by deciduous forest cover, soil organic carbon, electric conductivity, and moisture. The other omnivorous nematodes were on the negative side of axis 1. As for the abundance of herbivorous nematode taxa, since almost all of them were identified to genus level, their analysis was more detailed. *Helicotylenchus*, Neotylenchidae and *Rotylenchus* were on the quadrant positive for axis 1 and negative for axis 2, associated with deciduous forest cover, soil organic carbon, electric conductivity, albedo and pH. The distribution of Criconematidae seemed to be influenced by deciduous forest cover, soil organic carbon, electric conductivity and moisture. Interestingly, all of the other herbivorous nematodes were on the negative side of axis 1. *Heterodera*, *Meloidogyne*, *Xiphinema* and Trichodoridae were in the same quadrant, negative for axis 1 and positive for axis 2, and so these plant parasites of worldwide importance in agricultural settings all seemed to associate with apparent density, soil organic carbon and moisture. *Pratylenchus* was on the quadrant negative for both axis 1 and axis 2, influenced by apparent density, albedo, and pH (Figure 13A). There was no clear distinction in data distribution according to land use (Figure 13B) or protection status (Figure 13C), as 95% ellipsoids for datapoints in each group mostly overlapped with the others, but for the interconnection of the two factors (Figure 13D) there seemed to be a differentiation mainly on the protected native forests that were less overlapped with the other ellipsoids, they seemed to be less influenced by the variables as their datapoints were more concentrated in the origin of the CCA.

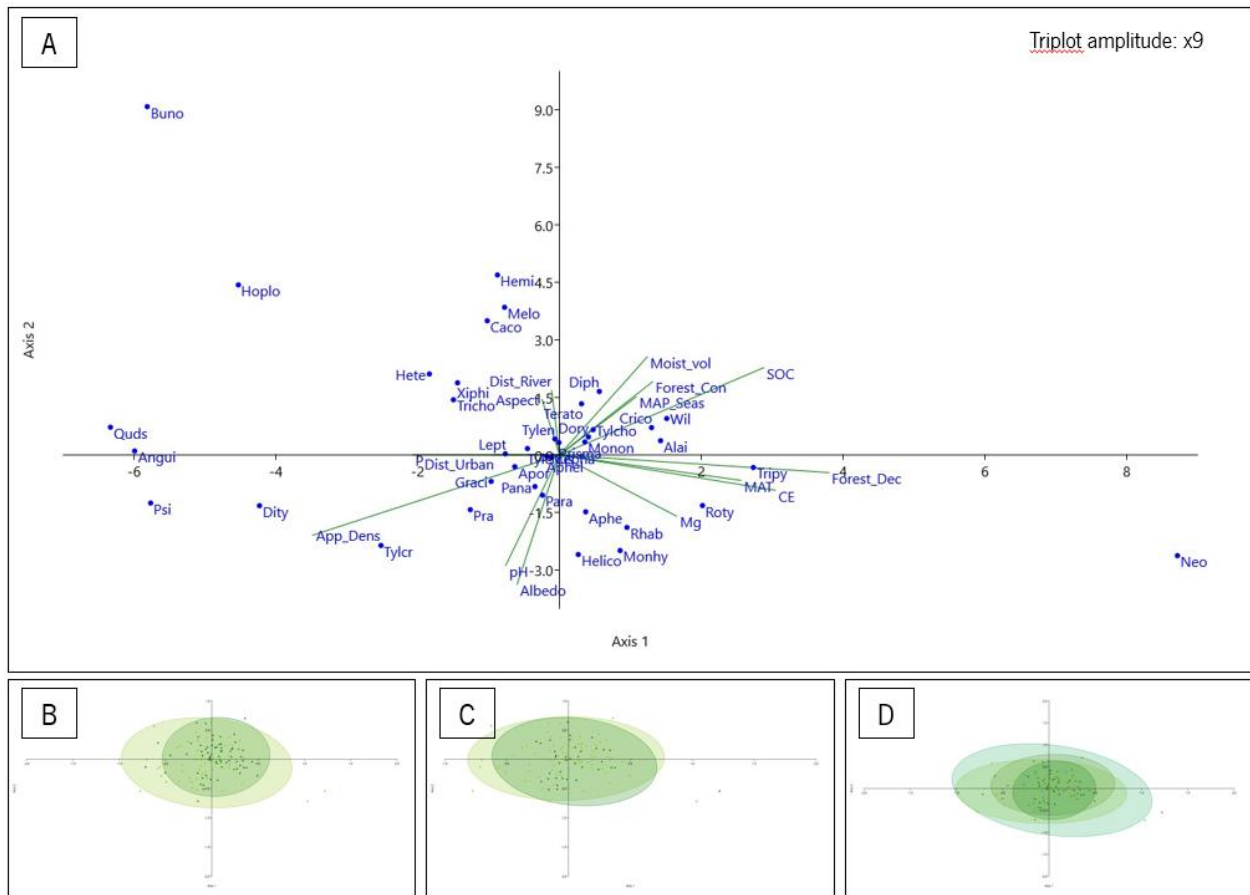


Figure 13 - Canonical correspondence analysis bi-plots of the influence of the environmental and edaphoclimatic variables on nematode families. Environmental variables are marked by arrows. Eigenvalues were 0.04276 and 0.03188 for the first and the second axis, respectively. (A) Canonical correspondence analysis biplot representing the environmental variable vectors and the nematode families (Triplot amplitude: x9). (B) overlapping of 95% ellipses for the land use groups. (C) overlapping of the 95% ellipses for the protection groups. (D) overlapping of the 95% ellipses for the group combinations of land use plus protection groups. Alai: Alaimidae; Buno: Bunonematidae; Monhy: Monhysteridae; Cepha: Cephalobidae; Pana: Panagrolamidae; Prisma: Pristomatolamidae; Rhab: Rhabditidae; Terato: Teratocephalidae; Wil: *Wilsonema*; Aphe: Aphelenchidae; Aphel: Aphelenchoididae; Angui: Anguinidae; Diph: Diphtherophoridae; Tylcho: *Tylencholaimus*; Tylen: Tylenchidae; Apor: Aporcelaimidae; Dory: Dorylaimidae; Lept: Leptonchidae; Quds: Qudsiianematidae; Tricho: Trichodoridae; Roty: *Rotylenchus*; Helico: *Helicotylenchus*; Neo: Neotylenchidae; Para: *Paratylenchus*; Pra: *Pratylenchus*; Tylcr: *Tylenchorhynchus*; Graci: *Gracilacus*; Crico: Criconematidae; Xiphi: *Xiphinema*; Hemi: *Hemicycliophora*; Tylchus: *Tylenchus*; Melo: *Meloidogyne*; Psi: *Psilenchus*; Caco: *Cacopaurus*; Hete: *Heterodera*; Dity: *Ditylenchus*; Hoplo: *Hoplolaimus*; Monon:

Mononchidae; Tripy: Tripylidae; Forest_Dec: Deciduous Forest Cover; SOC: Soil Organic Carbon; Forest_Con: Coniferous Forest Cover; MAP_Seas: Mean Annual Precipitation Seasonal; CE: Electric Conductivity; Moist_vol: Moist by volume of soil; Mg: Magnesium; P: Phosphorous; Dist_Urban: Distance to Urbanization; App_Dens: Apparent Density; Dist_River: Distance to Rivers; MAT: Mean Annual Temperature

4. DISCUSSION

4.1 Effects of land use and management on soil nematodes and soil ecosystem function

Land use affected soil biodiversity and functions, with soil from native forests having significantly greater enrichment and structure footprints and tending to have greater enrichment and structure indices. Taken together, these results suggest that native forests, unlike exotic forests, had a more fertile soil and a more complex community structure with more linkages in the food web, and therefore greater resilience (Ferris et al., 2001; Gao et al., 2020; Tomar and Ahmad, 2009). It was therefore unsurprising that native forests tended to have lower abundances of plant-parasitic nematodes ($p > 0.05$), which is here attributed to the capacity for natural regulation of pests and diseases of the soil food web. Forests ecosystems are already known for their overall structured and complex food webs, as reported in previous studies (Ferris et al., 2001; Zhang et al., 2015). Native forests also had a structure footprint larger than the enrichment footprint, which implies that soil communities in native forests rapidly use available nutrients, thus not having a great nutrient availability, and there was a greater investment in food web complexity and food chain length, which grant system stability. Exotic forests, however, even if they had a more nutrient depleted soil ecosystem and a more degraded community structure, had a more balanced enrichment: structure footprint ratio. This suggests that the activity and turnover rates of the enrichment indicators, representative of the prey, are sufficient to maintain the needs of the predators, the structure indicators, which suggests that their system is in metabolic balance (Ferris, 2010). Previous studies done with native pine tree (Yin et al., 2022) and exotic eucalyptus and acacia tree (Zhao et al., 2021) forest areas go with accordance to the results obtained in this study.

The metabolic footprints and abundance of trophic groups of free-living nematodes were significantly higher in native forest areas. Aboveground vegetation can affect the soil nematode community structure; therefore, the diversity and composition of plant species can be an important factor in determining the nematode communities. This can be especially important for forest ecosystems, since tree species are the main component of the forest (Kitagami et al., 2017). Plant species, soil type, soil fertility, litter depth, and forest management all influence the distribution and abundance of soil nematodes in forest ecosystems (Zhang et al., 2015).

Forest areas are characterized by having high cellulosic and lignified organic matter that fuels the fungal decomposition channel in soil food webs, hence their soils often have high C:N ratio, low pH and

fungus-dominated decomposition (Ferris et al., 2001; Neher et al., 2005). The correspondingly higher abundances of fungivore nematodes and fungus-dominated decomposition were also reported in previous studies in forest ecosystems (Keith et al., 2009; Kitagami et al., 2017) also putatively due to consumption of largely available mycorrhizal fungi (Cesarz et al., 2015) the low pH that results from the soil acidifications by litter accumulation (Kitagami et al., 2017), or because of large root biomass (Keith et al., 2009). In this study, there was a higher abundance of Aphelenchoididae (Fu2) followed by *Tylencholaimus* (Fu4), and since it was stated that Fu4 nematodes have feeding preferences for mycorrhizal fungi (Cesarz et al., 2015), I can presume that is one of the reasons why there was this fungus-dominated decomposition: these forests likely had a great abundance of mycorrhiza. All this supports the evidence found in this study, where it is possible to see that, in both native and exotic forests, there is high abundance of fungivore nematodes. I further speculate that due to the samples of this study being taken in autumn, when deciduous tree species, in this case study *Quercus robur* and *Quercus pyrenaica*, lose their leaves or even litter from pine, acacia or gum trees, causes a higher accumulation of litter, and maybe also because of the high root biomass present in the forests, not measured in this study.

In gum tree plantations, the magnitude of free-living nematode abundance has been shown to influence ecosystem processes and functions (e.g.: organic matter decomposition, nitrogen and phosphorus turnover and leaching and greenhouse gas emission). Low abundances of free-living nematodes may therefore indicate lower organic matter decomposition and nutrient turnover rates in this gum tree plantation in comparison to natural forests. Exotic forest areas have also been found to have lower nematode abundances in contrast to native forest areas, that have higher abundances (Gao et al., 2020). The lower nematode abundances in exotic tree species may be because, for example, gum tree litter is more complex and persistent to decompose than oak litter, and they release toxic compounds that inhibit the decomposition, and that reduces nutrient availability, supporting fewer nematodes (Zhao et al., 2021). The same was evident in this study, where exotic forests had significantly lower nematode abundances than native forests.

The low levels of herbivory we found in both native and exotic forests are in accordance with a study taken in managed and natural temperate forest (Čerevková et al., 2021), that found that herbivorous nematodes depend on the herbaceous undergrowth roots to feed, as they may prefer grass species with a high density of fine roots that favours their population growth (Zhao and Neher, 2013) because they are

more nutritious and easier to feed on than more lignified roots typical of trees (Čerevková et al., 2021). Although data on floristic diversity and coverage was not collected in the current study, it is possible that the low levels of herbivory may have been due to an overall low density of herbaceous stratum in the sampled forests. Furthermore, native forests tended to have a higher herbivory than exotic forests. One of the causes for the lower levels of herbivory in exotic forests may be related to the fact that these tree species, for example gum trees, have more complex and persistent components in their leaves (Zhao et al., 2021), and in native forests as well, pine trees release a considerable amount of needles (Keith et al., 2009), that sometimes gives no room for herbaceous undergrowth to develop and limiting the food resources for herbivorous nematodes. Another possible explanation for the low levels of herbivory may be that, because herbivorous nematodes have food preferences and their abundance and diversity depend on the plant species they are colonizing (De Deyn et al 2004) and the structure they are feeding on (Yeates et al., 2009), they may not have preference for any of the tree species or other possible vegetation that compose the native and exotic forests, however further research regarding this is needed. An explanation for the low levels of herbivory specifically on exotic and invasive tree species may be the natural enemy release hypothesis, that states that introduced invasive species in an exotic environment or region establish successfully if they left their co-evolved natural enemies behind (Keane and Crawley, 2002; Liu and Stiling, 2006). Exotic tree species in the analysed forests may have been released from their specific natural enemies, which would reduce their overall herbivory level. This was already stated in various studies, in invasive and exotic species (Keane and Crawley, 2002; Liu and Stiling, 2006) but further research regarding the findings in this study are needed to validate these speculations.

When addressing the effect of nature protection in soil biodiversity and functions, the nematode community indices, and metabolic footprints, were non-significantly higher in protected forest areas, except for the predator footprint. As well as the native forests, the greater enrichment and structure indices and footprints suggests that protected areas tended to have a more nutrient enriched soil system and a more complex community structure with greater resilience (Ferris et al., 2001; Gao et al., 2020; Tomar and Ahmad, 2009). This is in accordance with results on nematode abundances, since protected areas tended to have lower abundances of plant-parasitic nematodes and higher abundances of bacterivore nematodes in the soil system. It therefore appears to be a tendency towards positive effects of protected areas, but this is not significant.

When comparing the nematode alpha diversity between native and exotic forests, there was no significant difference between the two types of land use, however native forests tended to have a slightly higher alpha diversity. Greater diversity is usually associated with lower soil disturbance (Sanchez-Moreno and Ferris, 2018; Yeates, 2007). Native forest areas might have a tendency for lower disturbances, being more resilient and ensuring soil biodiversity and functions, possibly establishing more diverse soil communities through plant-soil feedback mechanisms. Plant-soil interactions can maintain biodiversity and functions (Fujii et al., 2018), they can increase soil fertility (Han et al., 2021), and restore ecosystems that have been modified by anthropogenic activities (Orozco-Aceves et al., 2015). Plant-soil feedback studies usually focus on enhancing aboveground plant biodiversity and less on belowground diversity. Normally, native plants have negative feedback in their habitat, and invasive and exotic plants have positive feedback because native plants co-evolved with their natural enemies, and exotic plants may not have their co-evolved natural enemies in the new region (Keane and Crawley, 2002; Liu and Stiling, 2006). However, I speculate that the higher diversity on native forests areas may be because oak trees litter, especially *Quercus robur* and *Quercus pyrenaica*, is easier to feed on than acacia and gum trees, so nematodes will have a preference towards the native forests culminating in a higher nematode diversity. Nematode alpha diversity was significantly higher in protected areas. Alas, studies of the soil biodiversity and function in protected forest areas are scarce and show limited to no effects of nature conservation on soil biodiversity (Ciobanu et al., 2019; Zeiss et al., 2022). Nematode analyses of Romanian grasslands assigned to Natura 2000, for example, showed no evidence of significant positive effects of protected areas on soil biodiversity and function (Ciobanu et al., 2019).

In what regards the first objective of this work: to investigate whether protected areas increase soil biodiversity and function in forest ecosystems, I can infer that current conservation measures are not having a significant effect on the soil system, but they tend to produce positive effects. Furthermore, native forests appear to be more sustainable and more important as a conservation target than exotic ones.

4.2 Influence of the environmental and edaphoclimatic variables on Soil Biodiversity and Function

In addition to the analysis taking into account nematode footprints, indices, diversity and trophic groups, an analysis considering nematode families and genera was done because, it has already been documented that analysis using a finer identification of nematodes gives better insights into the nematode

community structure and how it responds to environmental changes than the analysis of nematode trophic groups (Bhusal et al., 2014; Cesarz et al., 2015). However, contrary to expectations, this analysis did not provide more detailed information on the effects of environmental variables than what had already been established using nematode functions, as the variables that influenced nematode functions were the same that influenced nematode taxonomical diversity. Plant-parasitic nematodes, almost all of them identified to genus level, were represented in all quadrants of the CCA, which may reflect some lifestyle strategy or food preference that was not captured because of the lack of floristic data such as herbaceous stratum. *Heterodera*, *Meloidogyne*, *Xiphinema* and Trichodoridae, plant parasites of worldwide importance in agricultural settings (Chen et al., 2012; Estabraq and Liqaa, 2022; Oka et al., 2000), were in the same quadrant associated with apparent density, soil organic carbon and moisture, which implies that this quadrant brings together soil conditions that favour plant-parasitic nematodes with the ability to significantly reduce plant performance.

The variables that mostly influenced or affected, in general, the soil functional biodiversity were soil organic carbon, soil apparent density, soil moisture by volume, deciduous forest cover, and pH, and jointly had a low percentage of variance explanation, explaining 17% of the variance for the trophic groups, 16% for the nematode footprints, 15% for the nematode taxonomical diversity, and 15% for the indices and alpha diversity. The influence of these variables was already documented in various studies considering forest areas (Karuri, 2021; Keith et al., 2009; Zhang et al., 2012), and these low percentages of variance explanation were also documented by Archidona-Yuste et al. (2020), in plant-parasitic nematode community structure in cultivated olive tree stands. However, forest areas studies show higher explanation percentages, approximately 35% (Salamon and Wolters 2009; Zhang et al., 2012), but still variables explain only in part the nematode community structure (Salamon and Wolters 2009). These low percentages of variance explanation are indicative that some important variables were not considered, and further studies would be needed to elucidate on what other variables may have a more profound effect on nematode community structure. Furthermore, not only the percentage of explanation is small, but it is also not significant, except for the nematode trophic groups.

It is known that soil characteristics, such as soil organic carbon, pH, and electric conductivity are by far the most important factors driving nematode abundance at a global scale (van den Hoogen et al., 2019). Regions characterized by very low soil organic carbon stocks tend to have low nematode densities (van den Hoogen et al., 2019), and forest areas with low pH have a higher abundance of fungivorous

nematodes (Ferris et al., 2001). As for the moisture, nematodes reside in the water films surrounding soil particles (Brussaard, 1997) so, as expected, they will prefer environments with some humidity to it that facilitate their movement and feeding, rather than more dry environments (Costa et al., 2011). It is also stated that soil particles and porosity affect the movement and feeding of nematodes in soil (Quist et al., 2019), and I speculate, depending on the values of soil porosity, some larger nematodes, such as omnivores and predators, will be excluded, simply because they cannot move in between soil particles (Costa et al., 2011), so it is also expected that the soil apparent density, that is significantly correlated with porosity, will affect the abundance of larger nematodes, incidentally omnivores and/or cp 4-5 guilds, and therefore soil functions. The size exclusion limit would support the low abundances of omnivore and predator nematodes found in this study.

As described above, aboveground vegetation can affect the soil nematode community structure, and therefore the diversity and composition of plant species can be an important factor in determining the structure of nematode communities (Kitagami et al., 2017). The quality and quantity of litter inputs are likely to differ between tree species, with quality being a strong determinant of soil community structure. Broadleaf and coniferous tree species present a distinct contrast in litter quality, with coniferous leaf litter being more difficult to process than broadleaf litter (Keith et al., 2009). I anticipate that the pulse in organic matter in the soil caused by the accumulation of litter from the deciduous tree species may well have influenced the nematode abundance and functions. The diversity and composition of the aboveground plant species were not determined in this study, but our results suggest the percentage of deciduous forest cover may have influenced nematode community structure and functions. Therefore, further studies assessing conservation practices in forest ecosystems should consider the interrelationship between aboveground vegetation (dominance and diversity) and belowground biodiversity.

Since nematode abundances were used for the calculation of the indices, and metabolic footprints to assess soil biodiversity and function, it was expected, that the variables that influenced the nematode abundances, were going to affect its indices and metabolic footprints. An explanation for the lower percentages of variance explanations of environmental variables analysed may be because a large proportion of the unexplained variation is driven by features and mechanisms that were not considered, such as for example species interactions (Archidona-Yuste et al., 2020), microsite characteristics, such as microhabitat structure (Bhusal et al., 2014) and microsite temperature, soil structure (Salamon and Wolters 2009), elevation (Kergunteuil et al., 2016) and microbial biomass, that is essential for microbivores such as

bacterivore, fungivore, and omnivore nematodes, previously found to be one of the main factors determining nematode genera distribution (Zhang et al., 2012). In this study, we sought to interpret macroecological patterns, at the regional level, and further studies considering floristic diversity and elevation, for example, are needed to complement datasets and possibly increase the proportion of explained variance in this study. However, there may be differences and influences that are only noticeable on a finer scale, at microsite level, and research regarding that may complete the statements achieved in this study. A large dataset of observations on a wealth of soil taxa, their activities and functional measures will be made available by the SoilReCon Project for the exact sites, with determinations mostly done using the same original composite sample from where we derived most of our results. We expect this will allow for finer interpretations and framing of trends described here. More than climatic variables, other influences such as habitat destruction, fragmentation, and increasing population density may also be key driving forces for ecosystem change and should be minimized to increase forest ecosystem resilience (Leberger et al., 2020; Milad et al., 2011). Promoting connectivity of forest landscapes and habitat heterogeneity is likely to promote species survival, as well as changes in their composition and hence forest management (Milad et al., 2011), nature conservation should emphasize the idea of managing the ecosystems surrounding forest reserves. These variables were not taken into account in this study so further research regarding this is needed.

In what concerns the second specific objective of this work: to investigate to what extent protected areas increase soil biodiversity and function in forest ecosystems, I can infer that current conservation measures need to be improved to assure and increase soil biodiversity and function. Future research still needs to address plant-soil feedback/species interactions, possibly at a narrower scale, e.g., at a case-by-case analysis. Among the various locations sampled in this study, some of the forest areas sampled were plantations, even in native areas, where there was none to low coverage of undergrowth vegetation, that would certainly improve the soil system. In this study we concluded that deciduous forest cover was a variable with major influence on the soil biodiversity and functions, so I anticipate that integrating deciduous species in the protected forests could be a successful conservation practice. Planting more deciduous trees would turn exotic tree-dominated forests in protected areas into mixed forests. This has already been implemented, with mixed forests being better adapted to site conditions (Felton et al., 2016; Felton et al., 2010; Salamon and Wolters 2009). However, the extent to which ecological, economic, and societal benefits may rise from the conversion of monocultures to mixed forests depends on, for example, the tree

species being implemented and the bio-geographical region (Felton et al., 2010). Literature references could not be found on the conversion of gum tree or acacia monocultures to mixed forests using oak trees or pine trees, that are native to northern Portugal. If on one hand the plantation of pine trees in mixed forests can lead to positive outcomes for the ecosystem (Felton et al., 2016), on the other hand, reintroduction of a pine tree species (silver fir) produced no statistical differences in soil food web conditions, with silver fir reducing the metabolic activity and negatively affecting the abundance of several nematode trophic groups because of poor litter quality (Kondratow et al., 2019). So, it is not clear whether turning the gum tree and acacia monocultures into mixed forests using pine trees would be efficient to improve their environments, as they produce highly recalcitrant leaf litter. Given our results, I hypothesize interplanting oak trees in exotic forests, at a density and distribution to be determined, may increase the ecological and conservation value of these forests.

4.3 Conclusion

This study allowed us to respond to the objectives initially presented, that were whether and to what extent protected areas influences soil nematodes and function in forest ecosystems. We showed that Northern Portugal native forest areas have a more nutrient enriched soil system and a more complex community structure with a tendency for lower disturbances and, because of that, are more sustainable and better suited to ensure soil biodiversity and functions and should have a greater importance in nature conservation measures. As for the current work done by nature conservation in the North of Portugal, we showed that protected areas are not producing significant effects on the soil system, but there is a tendency towards positive effects. This highlights the importance of advising nature conservation policies on the potential positive effects on soil biodiversity and functions. These results also emphasize the importance of implementing significant changes to the protection of soils, because the above-ground biodiversity itself would also directly benefit from it. The study also showed that soil organic carbon, soil apparent density, soil moisture by volume, deciduous forest cover, and pH were the variables that mainly influenced or affected the soil functional biodiversity. However, further research is needed to complement, corroborate, or even contradict the speculations presented in this study, such as floristic diversity, and studies at the microsite level. We concluded that when establishing conservation areas, local soil biodiversity and soil monitoring should be considered. Perhaps if nature conservation polices begin to consider these types of factors that influence nematode abundances and the functions they perform in the North Portugal, it may

be a starting point to improve soil ecosystem conservation, not only on forest areas, but in all types of ecosystems. Here, we present evidence for a better belowground forest conservation that suggests that turning current monocultures of exotic species into mixed forests may imply significant positive changes to the preservation of soil communities. While such practices have already been successfully implemented in other regions, for the first time, we show their potential consequences for the conservation of soil nematodes.

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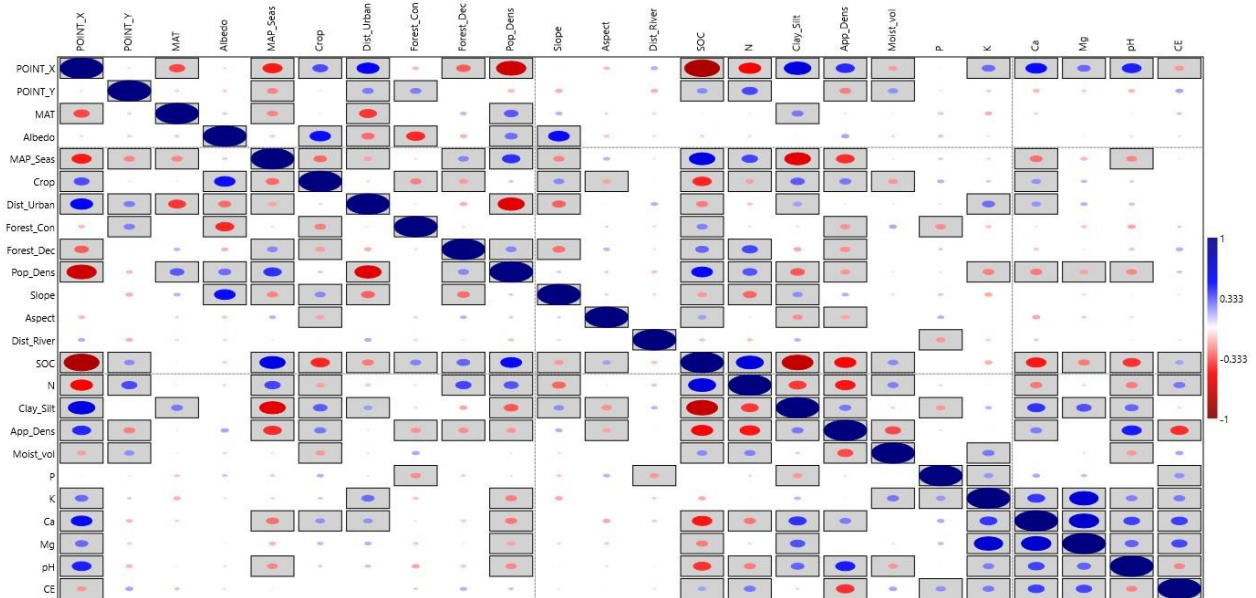
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6. ANNEXES



Annex 1 – Spearman correlations of the environmental and edaphoclimatic variables. The circles represented as blue, correspond to positive correlations, whereas the circles represented as red, correspond to negative correlations. Significant correlations are delimited in squares ($p < 0.05$).