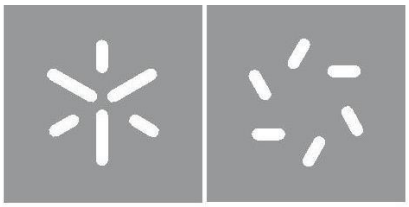
University of Minho
School of Sciences

Mariana Araújo da Cunha Sousa

Biodiversity and function in urban soils of the municipality of Braga

January 2025

Biodiversity and function in urban soils of the municipality of Braga
Mariana Araújo da Cunha Sousa
UMinho | 2025



University of Minho
School of Sciences

Mariana Araújo da Cunha Sousa

Biodiversity and function in urban soils of the municipality of Braga

Master thesis in Biodiversity, Ecology and Global Change

Work made under supervision of:
Doutora Sofia Rocha Costa

January 2025

DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

Este é um trabalho académico que pode ser utilizado por terceiros desde que respeitadas as regras e boas práticas internacionalmente aceites, no que concerne aos direitos de autor e direitos conexos.

Assim, o presente trabalho pode ser utilizado nos termos previstos na licença abaixo indicada. Caso o utilizador necessite de permissão para poder fazer um uso do trabalho em condições não previstas no licenciamento indicado, deverá contactar o autor, através do RepositóriUM da Universidade do Minho.

Licença concedida aos utilizadores deste trabalho



Atribuição-NãoComercial-SemDerivações CC BY-NC-ND

https://creativecommons.org/licenses/by-nc-nd/4.0/

Acknowledgments

Um agradecimento especial à minha orientadora, Doutora Sofia R. Costa, pela constante presença e apoio ao longo desta etapa. A sua dedicação, disponibilidade, paciência e conselhos foram fundamentais para a realização deste trabalho.

Ao meu grupo de laboratório, Rita Gonçalves, Carolina Duarte, Doutora Maria Teresa M.Almeida e em especial ao Rui Carvalho, por toda a grande ajuda na identificação dos nemátodes e na análise estatística.

Ao grupo de solos da Universidade de Coimbra, pela tão valiosa ajuda, em especial à Filipa Reis pela ajuda na identificação dos colêmbolos e à Dr. Marie Luise Carolina Bartz pelo enorme esforço e trabalho que teve na identificação das espécies de minhocas.

Ao Senhor Vereador Engenheiro Altino Bessa, pela autorização para aceder e proceder à recolha de amostras de solo dos espaços verdes do Município de Braga.

Ao Departamento de Biologia e CBMA e aos seus funcionários pela ajuda.

Uma grande obrigada àqueles que começaram como colegas de mestrado e acabaram por se tornar grandes amigos que sei que levarei para a vida, fizeram desta jornada uma etapa muito especial.

Obrigada a todos os meus amigos e família, em especial à minha mãe, pai, irmão e cunhada por estarem sempre comigo.

Por último, à Mariana e à Luísa, duas pessoas muito importantes na minha vida, pelo apoio incondicional, pelos conselhos intermináveis e pelas noites inesquecíveis que tornaram esta etapa da minha vida muito especial.

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Biodiversity and function in urban soils of the municipality of Braga

Abstract

The last century was marked by significant population growth, leading to rapid urbanization that has caused the degradation and fragmentation of natural habitats. Urban green spaces play a crucial role as substitutes for these lost natural environments, providing a range of ecosystem services that depend on soil and its biota. Understanding how urbanization affects belowground communities is necessary to assess and predict changes in ecosystem services provision. However, the full extent of urbanization impact on soil organisms remains unclear. The purpose of this project was to assess the impact of urbanization on the abundance, diversity, and functions of soil fauna to infer on soil ecological processes and quality across different urban land uses. Specific objectives were: (i) to integrate information from various faunal groups to analyse how land-use type affects soil communities and identifying which environmental variables are related to higher abundance, diversity, and magnitude of functions of soil organisms, (ii) to assess the relationship between diversity and the magnitude of a range of functions in urban ecosystems and (iii) to advance the soil ecology in urban environments to inform city management and planning decisions. To capture a representative selection of green spaces in the city of Braga, five land-use categories were sampled: unused lands, lawns, flower beds, forests, and roadsides. Physical-chemical parameters, plant cover, and biodiversity and function of faunal groups (nematodes, collembolans, mites, and earthworms) were assessed at each site. This study found that managed sites, such as lawns and roadsides, were nutrient-rich but disturbed, favouring bacterial over fungal decomposition, while flower beds were dominated by plant-parasitic nematodes. Non-managed sites, like forests and unused lands, generally displayed healthier ecosystems. However, even unused lands were dominated by plant-parasitic nematodes. Forests had the most balanced and stable ecological state, supporting healthier communities of nematodes, collembolans, and mites, while earthworms thrived in lawns and roadsides. The results also revealed that collembolans, mites, and nematodes shared preferences for similar environmental variables, including organic matter, legume cover, moisture, and site area. Earthworms, however, showed opposite preferences, favouring soils with high pH and exposed soil. Also, forests were the most effective land use in promoting ecosystem services. Finally, this study allowed for advancements in ecological theory in the soil component in urban environments, contributing to a better knowledge frame to inform city management and planning decisions.

Key Words: nematode community analysis, soil macrofauna, soil mesofauna, soil microfauna, urban green spaces

Biodiversidade e função em solos urbanos do Município de Braga

Resumo

O último século foi marcado por um elevado crescimento populacional, resultando numa rápida urbanização que degradou e fragmentou habitats naturais. Espaços verdes urbanos atuam como substitutos desses ambientes, fornecendo serviços de ecossistema dependentes do solo e da sua biota. Perceber como a urbanização afeta estes organismos é crucial para prever mudanças na provisão destes serviços. Contudo, o seu impacto na biota do solo ainda não é claro. O objetivo deste projeto foi avaliar o impacto da urbanização na abundância, diversidade e funções da fauna do solo para inferir processos ecológicos e qualidade em diferentes tipos de uso de terra. Objetivos específicos foram: (i) integrar dados de grupos faunísticos para analisar como o tipo de uso do solo afeta as comunidades e identificar quais variáveis ambientais estão ligadas a uma maior abundância, diversidade e magnitude de funções dos organismos, (ii) avaliar a relação entre diversidade e a magnitude de funções em ecossistemas urbanos e (iii) avançar a ecologia do solo em ambientes urbanos para apoiar a gestão e planeamento da cidade. Para capturar uma seleção representativa de espaços verdes na cidade de Braga, cinco categorias de uso da terra foram amostradas: terrenos baldios, relvados, canteiros, florestas e beiras de estrada. Avaliaram-se parâmetros físico-químicos, cobertura vegetal, biodiversidade e função de grupos faunísticos (nemátodes, colêmbolos, ácaros e minhocas). Este estudo conclui que locais geridos, como relvados e beiras de estrada, eram ricos em nutrientes, mas perturbados, promovendo a decomposição bacteriana em vez da fúngica, enquanto os canteiros eram dominados por nemátodes parasitas de plantas. Locais não geridos, como florestas e terrenos baldios, apresentaram ecossistemas mais saudáveis. No entanto, mesmo terrenos baldios eram dominados por nemátodes parasitas de plantas. As florestas apresentaram o estado ecológico mais equilibrado, suportando comunidades mais saudáveis de nemátodes, colêmbolos e ácaros, enquanto as minhocas prosperaram em relvados e beiras de estrada. Os resultados também revelaram que colêmbolos, ácaros e nemátodes compartilharam preferências por variáveis ambientais semelhantes, incluindo matéria orgânica, leguminosas, humidade e área. As minhocas, mostraram preferências opostas, favorecendo solos com pH elevado e exposto. Além disso, as florestas foram o uso da terra mais eficaz em promover serviços de ecossistema. Este estudo permitiu avanços na teoria ecológica na componente do solo em ambientes urbanos, permitindo informar decisões de gestão e planeamento da cidade.

Palavras-chave: análise da comunidade de nemátodes, espaços verdes urbanos, macrofauna do solo, mesofauna do solo, microfauna do solo

Index

Acknowledgments iii

Abstract v

Resumo vi

List of Figures x

List of Tables xiii

1. Introduction 1

1.1. Urban Growth and Urban Green Spaces as Substitutes for Lost Natural Environments 1

1.2. Functions of Soil Organisms in Urban Terrestrial Ecosystems 2

1.2.1. Soil Microbes 5

1.2.2. Soil Microfauna 6

1.2.3. Soil Mesofauna 7

1.2.4. Soil Macrofauna 8

1.3. Response of soil organisms to Urbanization 9

1.3.1. Soil Microbes 9

1.3.2. Soil Microfauna 9

1.3.3. Soil Mesofauna 10

1.3.4. Soil Macrofauna 11

1.4. Biodiversity and Function Across Land Uses in Urban Landscapes 11

1.4.1. Local Factors 12

1.4.2. Landscape factors 12

1.5. Objectives 13

2. Materials and methods 15

2.1. Sampling and data collection 15

2.1.1 Selection of sampling sites 15

2.1.2. Sample Collection 16
2.2. Assessment of Physical-Chemical Parameters 17
2.3. Assessments of biodiversity and function 18
2.3.1. Plant cover 18
2.3.2. Soil microfauna 18
2.3.3. Soil mesofauna 21
2.3.4. Soil macrofauna 24
2.4. Data Integration 25
3. Results 26
3.1. Effect of Land Use on Soil Organisms 26
3.1.1. Effect of Land use on Nematodes 26
3.1.2. Effect of Land use on Collembolans and Mites 32
3.1.3. Effect of Land use on Earthworms 34
3.2. Relationships between faunal groups 36
3.3. Influence of the environmental variables on soil organisms 37
3.3.1. Influence of the environmental variables on faunal Abundance and Biomass 37
3.3.2. Influence of the environmental variables on faunal biodiversity indices 39
3.3.3. Influence of the environmental variables on nematode community indices 40
3.3.4. Influence of the environmental variables on nematode metabolic footprints 41
3.3.5. Influence of the environmental variables on Collembolans and Mites parameters 41
3.3.6. Influence of the environmental variables on nematode taxonomical diversity 42
3.3.7. Influence of the environmental variables on earthworm taxonomical diversity 43
3.3.8. Influence of the environmental variables on Collembola morphotypic diversity 45
4. Discussion 46
4.1. Analysis of Soil Food Webs and Biological Soil Condition Across Land Uses 46
4.2. Effect of Land Use on Soil Communities 49

4.3. Influence of the environmental variables on soil organisms 52

4.3.1. Influence of the environmental variables on faunal biodiversity 52

4.3.3. Influence of the environmental variables on faunal taxonomical diversity 55

4.4. Contrasts and Interconnections in Soil Biota Indicators 56

4.5. Soil functions and Ecosystem services 58

4.6. Urban Management Strategies for Optimizing Ecosystem Services 59

4.7. Conclusions and future perspectives 61

5. References 64

List of Figures

Figure 1. Simplified food web showing trophic levels and connections between different functional groups of soil-dwelling organisms (adapted from Harkes, 2020). 3
Figure 2. Size distribution of soil organisms (adapted from Decaëns, 2010). 4
Figure 3. Illustrative photographs of selected categories of green space land-use types across the Municipality of Braga: (A) unused land; (B) lawn; (C) flower beds; (D) forest and (E) roundabouts/roadsides (photographs by the author). 15
Figure 4. Selected sites representing different types of land use in the Municipality of Braga. 16
Figure 5. Soil sampling design, illustrating a grid-like pattern of sampling points with a 15-meter spacing between them in both horizontal and vertical directions. 17
Figure 6. Nematode trophic groups based on the observation of mouth and upper digestive tract. (a) bacterivores, (b) fungivores, (c) herbivores, (d) predators and (e) omnivores. Figure credit: Ed Zaborski, University of Illinois. 19
Figure 7. Representative diagram of the characteristics associated with a soil-dwelling collembola and a collembola with high dispersal ability. Adapted from Carvalho, 2012. 22
Figure 8. External earthworm features used for identification (photographed by the author using a stereomicroscope). 24
Figure 9. Nematode density (A) and biomass (B) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$). 26
Figure 10. Nematode taxa diversity (Shannon index) (A); evenness (Pielou index) (B) and richness (Margalef index) (C) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$). 27
Figure 11. Nematode Maturity index (A); Enrichment index (B); Structure index (C); Basal index (D) and Channel index (E) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$). 29
Figure 12. Faunal profile representing the structure and enrichment conditions of the soil food web for the different land use types sampled in the city of Braga. A, B, C and D represent the different quadrats. 30
Figure 13. Nematode metabolic footprints as indicators of the magnitude of the ecosystem services performed by each nematode functional group sampled in the city of Braga for different land uses. Values are log-transformed means

of five replicates per land-use type (flower bed, forest, Lawn, Roadside, Unused Land). Asterisk denotes statistically significant differences among land uses for a given footprint. 31

Figure 14. Collembolan density (A) and mite density (B) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$). 32

Figure 15. Collembola morphotype diversity (Shannon index) (A); evenness (Pielou index) (B) and richness (Margalef index) (C) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. 33

Figure 16. Edaphic Collembola (A); Acari/Collembola (B) and mean trait value (C) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$). 34

Figure 17. Earthworm species abundance (A) and biomass (B) obtained from active searches in different land use types sampled in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$). 35

Figure 18. Earthworm species diversity (Shannon index) (A); evenness (Pielou index) (B) and richness (Margalef index) (C) obtained from active searches in different land use types sampled in the city of Braga. Values are median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$). 36

Figure 19. Canonical correspondence analysis of the influence of the environmental variables on nematodes, earthworms and collembolans Richness, Abundance and Biomass. Eigenvalues were 0.08451and 0.01639 for the first and the second axis, respectively. (A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and the nematodes, earthworms and collembolans Richness, Abundance and Biomass (Triplot amplitude: x1). (B) overlapping of 95% ellipses for the land use groups. Riq_Nem: nematode richness; Abu_Nem: nematode abundance; Bio_Nem: nematode biomass; Riq_Mi: earthworm richness; Abu_Mi: earthworm abundance; Bio_Mi: earthworm biomass; Riq_Col: Collembola richness; Abu_Col: Collembola abundance; Abu_Acari: mites abundance; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area. 38

Figure 20. Canonical correspondence analysis of the influence of the environmental variables on nematodes, earthworms and collembolans biodiversity indices. Eigenvalues were 0.08516 and 0.01082 for the first and the second axis, respectively. Canonical correspondence analysis bi-plot representing the environmental variable vectors and the nematodes, earthworms and collembolans Shannon, Pielou and Margalef indices (Triplot amplitude: x1). Shannon_Nem: nematode Shannon index; Pielou_Nem: nematode Pielou index; Margalef_Nem: nematode Margalef

index; Shannon_Mi: earthworm Shannon index; Pielou_Mi: earthworm Pielou index; Margalef_Mi: earthworm Margalef index; Shannon_Col: Collembola Shannon index; Pielou_Col: Collembola Pielou index; Margalef_Col: Collembola Margalef index; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area. 40

Figure 21. Canonical correspondence analysis of the influence of the environmental variables on nematode community indices. Eigenvalues were 0.0121 and 0.001593 for the first and the second axis, respectively. Canonical correspondence analysis bi-plot representing the environmental variable vectors and the Maturity, Enrichment, Structure, Basal, and Channel indices (Triplot amplitude: x1). MI: Maturity index; EI: Enrichment index; SI: Structure index; BI: Basal index; CI: Channel index; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.. 41

Figure 22. Canonical correspondence analysis of the influence of the environmental variables on nematode taxonomical diversity. Eigenvalues were 0.12 and 0.09364 for the first and the second axis, respectively. Canonical correspondence analysis bi-plot representing the environmental variable vectors and the nematode taxonomical diversity (Triplot amplitude: x2). Aphd: Aphelenchoididae; Aphe: Aphelenchidae; Diph: Diphtherophoridae; Tylm: Tylencholaimus; Pana: Panagrolaimidae; Ceph: Cephalobidae; Rhab: Rhabditidae; Tera: Teratocephalidae; Prsm: Prismatolaimidae; Wils: Wilsonema; Plec: Plectidae; Alai: Alaimidae; Heli: Helicotylenchus; Roty: Rotylenchus; Para: Paratylenchus; Prat: Pratylenchus; Melo: Meloidogyne; Tych: Tylenchorhynchus; Hoplo: Hoplolaimus; Xiph: Xiphinema; Grac: Gracilacus; Cric: Criconematidae; Caco: Cacopaurus; Hemi: Hemicycliophora; Tric: Trichodoridae; Quds: Qudsianematidae; Apor: Aporcelaimidae; Dory: Dorylaimidae; Mono: Mononchidae; Trip: Tripylidae; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area. 43

Figure 23. Canonical correspondence analysis of the influence of the environmental variables on earthworm taxonomical diversity. Eigenvalues were 0.7277 and 0.3439 for the first and the second axis, respectively. A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and earthworm taxonomical diversity (Triplot amplitude: x2). (B) overlapping of 95% ellipses for the land use groups. Drub: Dendodrilus rubidus; Bpar: Bimastos parvus; Otyr: Octolasion tyrtaeum; Ocya: Octolasion cyaneum; Olac: Octolasion cyaneumlacteum; Doct: Dendrobaena octaedra; Dhor: Dendrobaena hortensis; Drub: Dendrobaena rubidus; Smam: Satchellius mammalis; Aclo: Aporrectodea chlorotica; Acal: Aporrectodea caliginosa; Alon: Aporrectodea longa; Aros: Aporrectodea rosea; Atra: Aporrectodea trapezoides; Ldae sp1: Lumbricidae, Mdub: Microscolex dubius; Mpho: Microscolex phosphoreus; Acor: Amynthas corticis; Oct: Octodrilus sp; JUV: juveniles that cannot be identified at the family, genus, or species level; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area. 45

List of Tables

Table 1. Description of nematode ecological indices: maturity, enrichment, structure, basal, and channel indices. 20

Table 2. Description of enrichment, structure, bacterivore, fungivore, herbivore, omnivore, and predator metabolic footprints (according to Ferris, 2010). 21

Table 3. Characteristics and values of the characteristics used in the calculation of the eco-morphological index (EMI) value and for distinguishing different morphotypes (adapted from Santos, 2008 & Carvalho, 2012). 23

1. Introduction

1.1. Urban Growth and Urban Green Spaces as Substitutes for Lost Natural Environments

The last century was marked by a significant population growth, with over 55 % of the world population currently living in urban sites, a proportion that is expected to increase to 68 % by 2050 (United Nations, 2018). This rapid urbanization results in the degradation and fragmentation of the original natural habitats, along with their replacement with buildings and impermeable materials (e.g., asphalt, concrete), posing a major threat to biodiversity. Additionally, urbanization leads to heightened levels of noise, light, and chemical pollution, along with soil compaction (Fenoglio *et al.*, 2020).

Urbanization studies primarily focus on investigating the adverse effects of urbanization on ecosystems, biodiversity hotspots, or protected areas (Seto *et al.,* 2011; Güneralp & Seto, 2013). Typically, urbanization results in a reduction in biodiversity. As cities expand, not only the habitat of native species is destroyed but also habitats are created for a relatively few species that adapt to urban conditions. This process of replacing native species with increasingly widespread non-native species promotes biotic homogenization (McKinney, 2006), in other words, the composition of urban biomes is becoming more similar worldwide, a huge challenge to conservation (Lokatis & Jeschke, 2022). However, the urban landscape may hold high levels of biodiversity within cities, and the effects that urban biodiversity has on ecosystem services, including human well-being, cannot be ignored (Fuller *et al.*, 2007). Some taxa experiencing global decline often thrive in urban habitats, exhibiting high population densities (Goddard *et al.* 2010). This is the case of urban parks in San Francisco, USA, that have been shown to support higher mean abundances of bumblebees (*Bombus* spp.) than two parks beyond the city boundary (McFrederick & LeBuhn, 2006). Another example was found in the UK, where populations of common frog *Rana temporaria* have experienced declines in rural areas but increase in urban parks and gardens (Carrier & Beebee, 2003). Most research has addressed aboveground biodiversity, and there is limited knowledge concerning how belowground organisms respond to urbanization.

Urban green spaces play a crucial role as substitutes for the lost natural environments (Francini *et al.*, 2018). A healthy and sustainable urban green space provides an extensive range of ecosystem services, including carbon and nitrogen sequestration (Setälä *et al.*, 2017), stormwater interception and purification (Valtanen *et al.*, 2015), and biodiversity and climate regulation (Bolund and Hunhammar, 1999). These ecosystem services rely exclusively on the soil, and consequently on the soil biota (Francini *et al.*, 2018).

Given their pivotal role in ecosystem functionality, understanding how urbanization impacts the belowground communities is necessary to assess and predict alterations in the provision of ecosystem services, strictly dependent on biodiversity in urban areas (Ziter, 2016). Yet, the extent to which biodiversity of different soil organisms is associated with multiple dimensions of ecosystem functioning in urban green spaces remains unknown (Fan *et al.,* 2023).

1.2. Functions of Soil Organisms in Urban Terrestrial Ecosystems

Soil communities are extremely complex and diverse, with millions of species and billions of individual organisms being found within a single ecosystem (Bardgett & van der Putten, 2014). Soils offer a vast variety of habitats for these communities, arranged in a highly organized combination of a solid mineral phase, a network of water and air-filled pores, and dead decomposing organic matter. In this compact environment, life is concentrated within a network of pores that typically comprises 30 % to more than 60 % of the soil volume in the upper horizons. Living and moving in soil pores is the first important obstacle for soil organisms. Pore size is highly diverse, and the available space theoretically accessible to a given organism can be decreased by the amphibious nature of the system, partly filled with water and partly with air. As so, soil organisms should be primarily adapted to this important double constraint (Wurst *et al.,* 2012). Another important constraint in soil is feeding on mainly low-quality food, as a large proportion of soil organisms are detritivores or decomposers feeding on litter. The most abundant resources are leaf and woody litter deposited at the soil surface, root material, and soil organic matter. Despite relatively abundant, these elements are mostly of a low nutritive quality and difficult to digest (Lavelle & Spain, 2001). Soil organisms have evolved different kinds of adaptive strategies to deal with these three major constraints of the soil habitat: size exclusion, air: water proportions, and litter nutritional quality. However, none of the groups of soil organisms is optimally adapted to all three main soil constraints, which has led to the development of associations in which complementary adaptive strengths are shared.

Ecologists first began to explore the importance of trophic interactions in soil for ecosystem processes in the early 1980s. Microcosm studies during this period unveiled their role in stimulating processes of decomposition and nutrient mineralization, and plant nutrient acquisition and growth (Anderson *et al.* 1983; Ingham *et al.* 1985). Although each group of organisms individually plays a specific role in the functions and properties of soil, they do not exist in isolation, in fact, their diversity and interactions are responsible for the soil functioning (Jeffery *et al.*, 2010), forming part of a complex network, the soil food web (Figure 1) (Richter

et al., 2019). A structured food web that enables energy flow is characteristic of a healthy and functioning soil ecosystem, playing a crucial role in providing essential ecosystem services (Kibblewhite *et al.*, 2008).

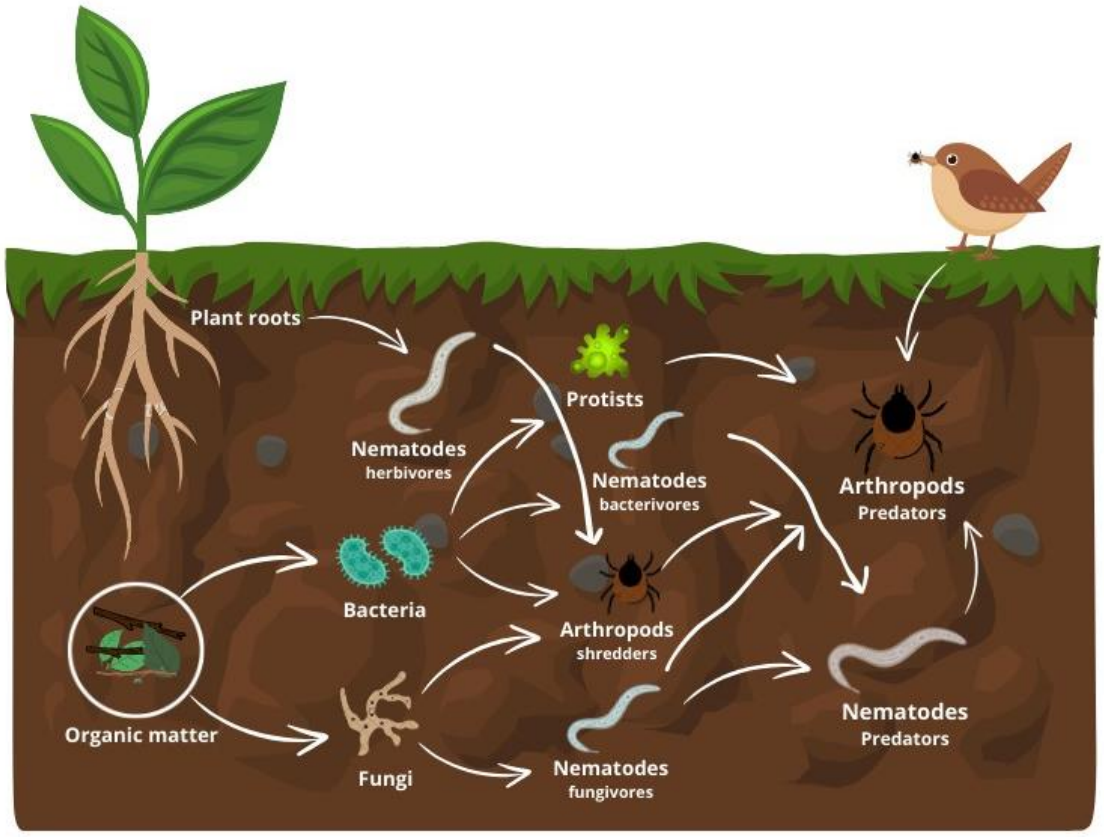


Figure 1. Simplified food web showing trophic levels and connections between different functional groups of soil-dwelling organisms (adapted from Harkes, 2020).

Plants, as primary producers, combine mineral nutrients from the soil with atmospheric carbon forming organic compounds through photosynthesis, initiating what is called the "green" food chain. Plants are then consumed by herbivores which, in turn, are consumed by higher trophic level organisms (Gan & Wickings, 2020). However, a significant portion of this plant biomass, almost 90%, is never consumed by herbivores, and ultimately returns to the soil as decomposing organic matter, which serves as the foundation of a parallel food chain, known as decomposition or the "brown" food chain (Gessner *et al.,* 2010). Organic matter, as litter, can be broken down in small particles by grazers and detritivores, transported and mixed through soil by ecosystem engineers and then digested by the microbial community, bacteria and fungi. Both groups of microbiota, through extracellular digestion, secrete enzymes that break down and solubilize

complex molecules, including the most recalcitrant substrates (such as cellulose, chitin, lignin, or phenol protein compounds) for subsequent uptake. Fungi have a broader and more specialized array of enzymes capable of decomposing all sorts of substrates including nutrient-poor molecules with a large C:N ratio. Their hyphae also allow them to infiltrate, fragment and access more stable organic matter (Allison, 2006), making the fungal decomposition channel associated with humus content, and more stable soil conditions. On the other hand, with their rapid turnover rates, bacteria rapidly colonize and exploit "easy" resources, as root exudates and excretions from other organisms, or even "stolen" fungal-digested material. This way, the bacterial decomposition channel is associated with more unstable and enriched conditions (Costa, 2024). The resulting materials of decomposition, as well as microbiota themselves, are then consumed by other organisms throughout the soil food web (Lattaud *et al.*, 1999).

The size of the organism largely reflects the ability to face each kind of soil constraint. Soil-dwelling organisms are generally grouped according to their size: <100 µm body size for the microbes (e.g. bacteria, archaea, and fungi) and microfauna (e.g. protozoa, nematodes), to mesofauna with body size between 100 µm and 2 mm (e.g. collembolans and mites), macrofauna with body size >2 mm (earthworms, myriapods, and insects) and up to megafauna with body size >2 cm (e.g. moles, voles) (Swift *et al.,* 1979) (Figure 2).

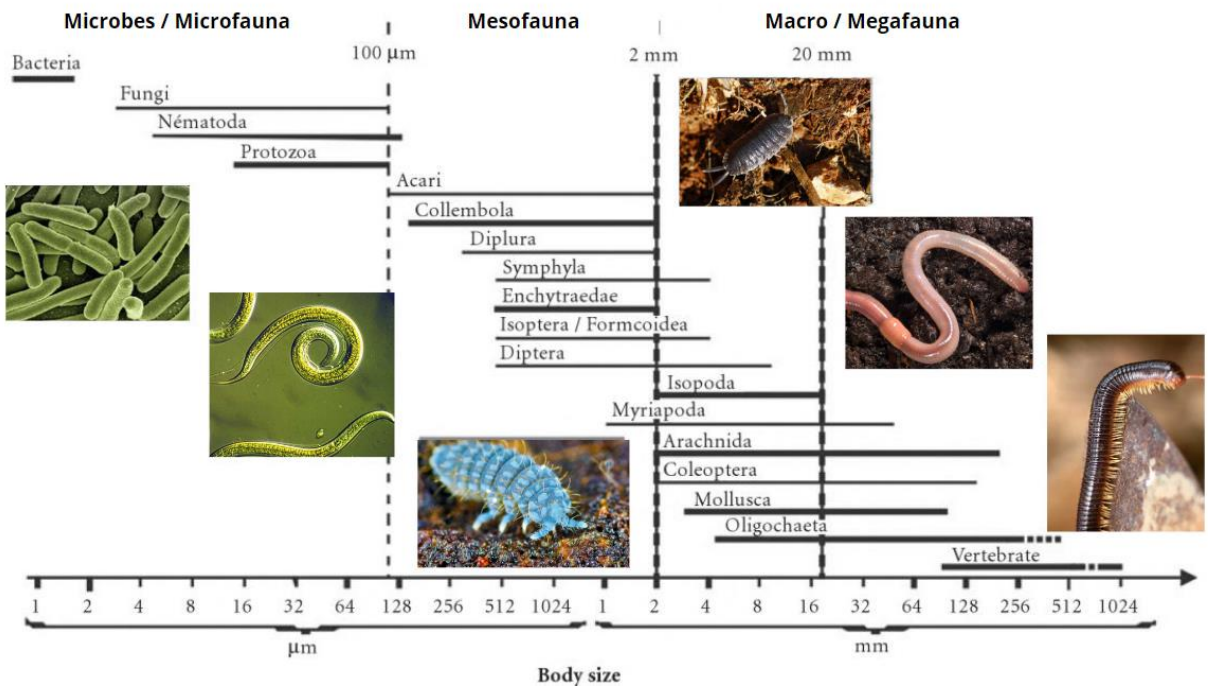


Figure 2. Size distribution of soil organisms (adapted from Decaëns, 2010).

Soil biodiversity is positively correlated with multiple dimensions of ecosystem functions in urban spaces. Urban spaces with greater soil biodiversity support higher levels of key groups of functions such as: decomposition of organic matter and, thus, regulation of nutrient cycling; fixation of atmospheric nitrogen, making it available for plants; degradation of anthropogenic compounds like pesticides; stabilization of soil aggregates, specifically through the building of clay–humus complexes; improvement of soil structure and porosity as a result of burrowing activities; influencing of soil pH by nitrification and denitrification, resulting in mobility changes of heavy metals and pest control (Beck *et al.* 2005). Human health is directly influenced by soil biodiversity, as it not only provides disease control but also influences the quantity and quality of the food we eat, the air we breathe and the water we drink (Wall *et al.,* 2015). Given the key role of soil biodiversity in maintaining these ecosystem functions, a detailed analysis of the role of soil biodiversity in regulating ecosystem services is crucial to gain a deeper insight on the potential consequences of soil biodiversity losses for the maintenance of multiple ecosystem functions and services that are critical for human wellbeing and global ecosystem sustainability (Delgado-Baquerizo *et al.*, 2020).

Each faunal group has been explored for their indicator value, taxonomical and functional diversity (Wall *et al.*, 2012). Due to their ubiquity, importance in the ecosystem, and sensitivity to anthropogenic pressures, these faunal groups are used as bioindicators, offering a quick and cost-effective way to assess soil quality (Beck *et al.,* 2005). More recently some of them have been used to evaluate the effect of urbanization in urban biodiversity (e.g. Chen *et al.*, 2021; Gong *et al.,* 2023; Tóth *et al.,* 2020). Additionally, the diversity of key individual soil organisms supports different aspects of ecosystem functions and services (Fan *et al.,* 2023). Consequently, the biodiversity of soil organisms across multiple trophic levels may be necessary to explain numerous aspects of ecosystem functions and services provided by urban green spaces (Delgado-Baquerizo *et al.*, 2020). Therefore, conducting an analysis of the various faunal groups - microbiota, microfauna, mesofauna and macrofauna - can return information on the impact of urbanization in different groups of functionally important soil organisms, whilst providing an assessment on the impact of urbanization in the different soil components (Szabó *et al.*, 2023).

1.2.1. Soil Microbes

Soil microbes - bacteria, archaea, and fungi - are the most abundant and diverse groups of soil organisms. One gram of soil may harbour up to 10 billion microorganisms of possibly thousands of different species (Rosselló & Amann, 2001). Bacteria and archaea are the smallest independently living, single-celled

organisms on earth, that may occur as cocci, rods, or spirals, and some bacteria common in soils, such as the Actinomycetales, can form branching filaments. Fungi are eukarya and more directly related to plants and animals than to bacteria or archaea. While some fungi occur as single-celled organisms, typically known as yeasts, many grow as hyphae, which are cylindrical thread-like structures, that intertwine to form a mycelium, the main body of the fungus (Aislabie et *al.,* 2013). In soils, microbes play a pivotal role in nutrient cycling. They play fundamental roles in cycling carbon, nitrogen and phosphorus, which are essential for producing biomolecules such as amino acids, proteins, DNA and RNA, the fundamental compounds of life. They are also vital for provisioning and regulating other services provided by soil such as physical support, as microbial products formed during nutrient cycling and organic matter production are critical to soil aggregation; raw materials, since soil microbes produce antimicrobial agents and enzymes used for biotechnological purposes; carbon storage and regulation of greenhouse gas emissions; plant growth promotion and are also responsible for biological control of pests, weeds and pathogens (Dominati *et al.,* 2010). Therefore, investigating how microorganisms respond to urbanization is particularly relevant given their fundamental roles in maintaining ecosystem functions and services (Bardgett & van der Putten, 2014). Furthermore, soil microorganisms also have direct impact in human health and well-being (Rook, 2013); the beneficial health effects of being close to nature was suggested to be due to the build-up of the antigenic repertoire of the immune system by exposure to microbial diversity (Stein *et al.*, 2016). This is especially relevant to urban residents, whose primary exposure to nature typically occurs within green spaces. However, how soil microbial diversity and community composition react to urbanization remains scarcely understood and idiosyncratic (Liu *et al.*, 2022). Indeed, their communities may be more representative of long-term land use intensity and vegetation cover than of short-term changes, especially as these organisms can remain in dead or quiescent forms in soil for a long time (Boeddinghaus *et al.*, 2019).

1.2.2. Soil Microfauna

Among microfauna, nematodes have been a valuable tool for assessing the structure, function, and resilience of the soil food web (Du Preez *et al.*, 2022). They are a dominant component of the soil community and are by far the most abundant animals on Earth (Van Den Hoogen *et al.*, 2019). Nematodes live in most terrestrial habitats, occupy key positions at most trophic levels in soil food webs due to their various feeding habits and as food resources for other organisms (Bongers and Ferris, 1999), including in urban soils (e.g. Pavao-Zuckerman *et al.*, 2007). Nematodes have essential roles in soil processes and ecosystem functions.

Through their metabolic and behavioural activity, nematodes contribute to plant growth and primary productivity, carbon and nutrient cycling, as well as pest and disease control. Nematodes also indirectly contribute to decomposition of soil organic matter by regulating populations of decomposer fungi and bacteria (Ferris, 2010; Yeates *et al.,* 2009). Their functional role in soils can be inferred by their trophic position, being often categorized into trophic groups according to their feeding habits: bacterivores, fungivores, herbivores, omnivores and predators (Yeates *et al.*, 1993). However, the link between ecosystem functions and trophic groups is not always straightforward or clear, as different trophic groups can be involved in the same function; for example nematodes from all trophic groups contribute to the carbon transformation or nutrient cycling, directly or indirectly (Lazarova *et al.,* 2021). Another representation of nematode functional diversity is related to their life history strategies. Pure colonizers or r-strategists, reproduce rapidly, produce many small eggs and quickly exploit nutrient-rich habitats, whereas persisters or k-strategists produce few but big eggs and hardly react at transient conditions of high food availability (Bongers & Bongers, 1998). The colonizer-persister life strategy of nematodes can be categorized on a CP scale ranging from 1 to 5: nematodes classified as cp-1, the enrichment opportunists, prosper under nutrient enrichment conditions and are relatively tolerant to pollution-induced stress; cp-2 generalist opportunists occur under food-rich as well as food-poor conditions and are very tolerant to pollutants and other disturbances; cp-3 nematodes are relatively susceptible to disturbances; cp-4 nematodes are sensitive to pollutants; and the cp-5 persisters are very sensitive to pollutants and other disturbances (Bongers & Bongers, 1998). In summary, colonizer nematodes with lower cp values (cp1 and cp2) are opportunistic nematodes indicating resource availability or disturbance, in contrast, persister nematodes with high cp values (cp4 and cp5) are indicators of system stability, food web complexity and connectance (Ferris *et al.*, 2001; Sánchez-Moreno and Ferris, 2018). Therefore, to reflect a more stable system with a more complex food web, the soil must have a higher proportion of higher cp value nematodes rather than lower cp value nematodes. The combination of trophic groups with life strategies resulting in nematode functional guilds have since formed the basis for nematode community analyses. Nematode-based ecological indices and metabolic footprints have been aiding the interpretation of soil ecology in several studies across biomes and land-use types (reviewed in Du Preez et al., 2022).

1.2.3. Soil Mesofauna

Soil mesofauna is mostly composed of the taxa: Acari, Collembola, Tardigrada, Protura, Diplura and Enchytraeidae (Coleman, 2008). Acari, or mites, have a hard body and eight legs. Collembola, or springtails

are primitive insects, with six legs and antennae, but no wings. Both mites and collembolans inhabit air-filled pores and litter layers, usually found in more superficial soil, and the number of known collembolan species is much lower than that of the mites, but they may reach the same abundances. Mite species are found in different trophic levels, from herbivores, bacterivores, fungivores and predators, while collembolans are more restricted in their diet, as most species feed on fungi and algae, but a few feed on plants or are predators (Petersen & Luxton, 1982). Due to their abundance and species richness, and their nearly ubiquitous presence in soils, mites and collembolans have been proposed as soil quality indicators (Bokhorst *et al.,* 2008; Parisi *et al.,* 2005). Collembolans and mites communities play a vital role in soil ecosystem health because as detritivores, they have important roles in promoting decomposition, nutrient cycling, carbon sequestration and soil formation (Filser, 2002; Seastedt and Crossley, 1980). They are also relevant in suppressing pests and diseases, and serve as food for other organisms, while simultaneously regulating bacterial and fungal populations (Coleman, 2008). As so, it is crucial to perceive how the mite and collembolan communities would respond to the environmental changes induced by urbanization (Yu *et al.*, 2021), however, they remain scarcely studied in urban ecosystems. Collembolans present a diverse array of eco-morphological and life-history traits (Hopkin, 1997; Parisi *et al.*, 2005), a variety of different life-forms related to vertical distribution in soils and dispersal ability (Ponge *et al.*, 2006). They can be classified in three eco-morphological groups, which indicate their levels of adaptation to the soil: edaphic, with morphotypes more adapted to the soil; hemi edaphic, with intermediate adaptation; and epigeic, with morphotypes more adapted to litter (Vandewalle *et al.*, 2010; Silva *et al.*, 2016).

1.2.4. Soil Macrofauna

Earthworms form the dominant component in the animal biomass of the soil. They belong to the phylum Annelida, consisting of coelomate animals that have their body divided into a series of compartments or segments (Sims & Gerard, 2023). Among soil macrofauna, earthworms are considered "soil ecosystem engineers", as they intermix soil resources and create habitats for other soil organisms through bioturbation (Blouin *et al.,* 2013). Earthworm bioturbation produces two kinds of soil structures: burrows, produced by excavation or by pushing the soil aside; and casts produced by ingestion of soil and organic matter, then excreted on the soil surface or below-ground (Lee & Foster, 1991). As so, they play crucial roles in establishing and sustaining soil structure by building pore networks and also have impact on nutrient cycling, improving soil aggregation by mixing mineral and organic particles in their digestive tract (Milleret *et al.*, 2009), thus

they are considered efficient bioindicators of soil health (Valchovski, 2010). Despite the important role earthworms play in soil processes and functions, few studies have focused on their community patterns in disturbed systems, especially in an urban environment (Xie *et al.*, 2018). Earthworms can be divided into different ecological groups based on their physiology, feeding and burrowing behaviour: epigeic earthworm species inhabit and feed on the surface litter; anecic species create deep vertical burrows in the soil but browse on the soil surface, playing a crucial role in the burial of surface litter; and endogeic species burrow horizontally and feed mainly in the rhizosphere and subsoil (Lee, 1985).

1.3. Response of soil organisms to Urbanization

Recently, studies have been conducted to analyse the impact of urbanization on different soil faunal groups in various sites worldwide.

1.3.1. Soil Microbes

Across an urbanization gradient, both soil bacterial/archaeal and fungal community exhibit significantly different composition (Chen *et al.,* 2021; Wang *et al.,* 2017; Liu et *al.,* 2022). However, the effects of urbanization can vary among microbial taxonomic groups. If soil fungal richness seems to be lower in highly urbanized locations, soil bacterial/archaeal richness may not be affected by urbanization (Epp Schmidt *et al.,* 2017; Chen *et al.,* 2021). However, contrasting studies reported that fungal richness and community composition did not differ with urban stress, whereas bacterial community composition did (Reese *et al.,* 2016). Furthermore, urbanization can also alter soil microbial carbon and nutrient cycling by shaping the communities of microorganisms found in urban habitats. Ammonia-oxidizing bacteria were more abundant in highly urbanized locations, and ammonia-oxidizing archaea were more abundant in lowly and moderately urbanized sites. However, in a similar study, the ammonia-oxidizing archaea abundances were higher in the urban soils compared to the suburban soils (Wang *et al.,* 2017). As so, despite the results of these different studies indicating urbanization can significantly impact and shape microbial community diversity and function, the full impact of urbanization on soil microbial communities remains unclear and requires further research in different urban ecosystems around the world.

1.3.2. Soil Microfauna

Nematode communities reflect changes in terrestrial habitats due to their rapid response to environmental and anthropogenic disturbances (Du Preez *et al.*, 2022). A wealth of literature has documented

soil nematode community responses in a wide range of habitats and ecosystems, however there are only few studies on the relationships between urbanization and soil nematode diversity. Similar nematode richness has been detected in urban parks and surrounding forests, exceeding that in urban residential areas and surrounding farmlands. Nematode communities in parks and residential areas were, however, more homogenous than in forests and farmlands. Taken together, the results suggest that urbanization processes reduce nematode diversity, with e.g., 30% loss in residential areas compared to forests, and homogenize soil nematode communities (Gong *et al.*, 2023). However, in a similar study, the diversity of nematodes was not affected by urban land use. Yet, there tended to be functional differences in the nematode communities along a land use gradient. A comparison of functional group abundances indicates that urban soils tend to have lower abundances of higher trophic levels (predators and omnivores) (Pavao-Zuckerman & Coleman, 2007). This is in accordance with a recent study that found that the proportions of omnivores (by 45-60%) and predators (by 40-50%) as well as temporal ecosystem stability (by 10-20%), were reduced in urban ecosystems, while the proportions of fungivores and bacterivores increased (30-60% and 10-20%, respectively) (Gong *et al.*, 2024). As so, further analysis in various urban ecosystems worldwide is needed to fully understand the impact of urbanization on nematode communities.

1.3.3. Soil Mesofauna

The increase in soil pH, total nitrogen content, and heavy metals, typical of urbanized areas, have been shown to reduce the soil mesofauna (collembolan and mite communities) abundance and diversity. Although a significant relationship between soil mesofauna community and impervious surface in urban soils could not be attributed to landscape changes, the site area was positively correlated with soil mesofauna abundance; a decrease of habitat area is a consequence of habitat fragmentation due to urbanization (Yu *et al.,* 2021). Long-term exposure (30 years) to urbanization has resulted in erosion in collembolan diversity and the formation of species-poor communities, species replacement, loss of specialized forms, whilst promoting the invasion by exotic species in urban green spaces (street lawns and park lawns). More resistant collembolan communities were found over time in less stressed urban greening components such as park lawn soils compared to street lawn soils, as although time significantly decreased the values of richness and diversity for the entire urban green and street lawns, the differences were not significant for collembola communities from park lawns (Rzeszowski & Sterzyńska, 2016). Regardless, further research is needed to better understand how urbanization affects mesofauna.

1.3.4. Soil Macrofauna

Both the abundance and biomass of earthworms in residential metropolitan areas have been found to be relatively low. Earthworm communities were positively correlated with soil moisture and there was a sharp decrease in earthworm abundance with decreasing pH levels, as under low soil pH environment, the lack of nutrients (e.g., calcium) which are necessary for earthworm survival can result in the reduction of earthworm abundance. Furthermore, the influence of the urban landscape, as the percentage of impervious and green space surface, the amount of landscape cover types, patch density and landscape fragment were also perceived to significantly affect the earthworm community patterns (Xie *et al.*, 2018). Earthworm communities are significantly structured by habitat type, as despite differences in climate, soils and biogeography among the cities, earthworm communities exhibited high similarity within the urban habitat types. This homogenization in urban habitats is presumably due to human-facilitated dispersal (Tóth *et al.*, 2020). Nevertheless, additional research is necessary to gain a clearer understanding of how urbanization impacts earthworms.

1.4. Biodiversity and Function Across Land Uses in Urban Landscapes

Studies of intra-urban biodiversity levels frequently focus on changes in species richness, measures of species diversity and species abundance along urban–rural gradients (e.g. Niemelä & Kotze, 2009; Martinson et Raupp, 2013). This gradient is demarcated from the border of a city towards the city centre and is frequently used as the sole explanatory factor influencing the biodiversity of urban areas (Beninde *et al.*, 2015). Although practical, this approach tends to oversimplify the urban landscape, leading to ambiguous interpretations of results (McDonnell & Hahs, 2008). Urban sites are characterized by their heterogeneity, presenting a diverse mixture of land uses within a relatively small geographic area. This heterogeneity is hardly captured by a simple gradient approach that may mask the underlying driving factors of the biophysical urban landscape (Beninde *et al.*, 2015). Therefore, to understand the impact of urbanization in the biological communities, it is necessary to conduct comparative studies of biodiversity and function among different land uses, since the soil organisms of these different spaces may suffer different impacts from urbanization, according to the individual factors that they present. Ecological studies often distinguish local habitat features from those of the surrounding landscape (Ricketts, 2001). While local factors establish habitat suitability (in terms of species survival), landscape factors regulate the permeability of the surrounding landscape to species dispersal (Beninde *et al.*, 2015).

1.4.1. Local Factors

The local factors of a certain habitat, as plant cover pH, moisture, organic matter, and the management practices associated with these, are consistently considered key factors that determine the diversity of soil communities in urban environments (Guilland *et al.*, 2018). Plant diversity was shown to indirectly promote soil multifunctionality in city parks via changes in the biodiversity of soil fauna (Schittko *et al.,* 2022), by forming symbiotic systems (e.g. mycorrhizal plant-arbuscular mycorrhizal fungi, mycorrhizal plant- ectomycorrhizal fungi, plant- diazotrophs) or indirectly influencing soil biodiversity by altering soil conditions (Fan *et al.*, 2023). Also, evaluating the proportion and percentage of plant cover allows the assessment of the overall biodiversity and ecological health of an area. For example, a high proportion of legumes suggests a healthy nitrogen-fixing plant population, which is beneficial for soil fertility and supports a diverse range of organisms (Temperton *et al.*, 2007). Urban soils are often characterized by an increased soil pH compared to natural habitats (Jim, 1998; Pouyat *et al.*, 2015; Asabere *et al.*, 2018), as a result of artificial product wastes (e.g. broken bricks) that are buried into soils during city constructions and residential daily life in urban areas. A range of studies suggest that alkalinization in urban soils has the potential to modify the soil communities, as soil pH affects microbial survival and fitness in urban ecosystems (Liu et *al.,* 2021). This was also shown for other soil organisms including earthworms, whose abundance gradually decreases with pH within the neutral range (Xie *et al.*, 2018). In managed urban green spaces, irrigation and fertilization may substantially change soil characteristics, leading to high levels of moisture and organic matter, respectively (Yu *et al.*, 2012). These changes in soil parameters, that ultimately depend on vegetation cover and management in these ecosystems, impact soil biota. For example, abundance and biomass of the earthworm community is positively correlated with soil moisture, as low soil moisture can reduce earthworm weight and decrease their survival rate if they are under challenging conditions (Xie *et al.*, 2018). Moreover, the increase in organic matter content is expected to favour microbes, thereby boosting the population growth of microbial consumers, as for example microbivore nematodes, and contributing to their diversity (Neilson *et al.*, 2020).

1.4.2. Landscape factors

Not only local factors influence local diversity and abundance, but landscape factors can also have a large impact on organisms: the area of habitat fragments, distance to natural areas and habitat connectivity may affect the biological community (Otoshi *et al.*, 2015). Cities typically contain small, fragmented, and

isolated habitat patches. As a result, the usual positive associations between biodiversity, area, and connectivity are particularly relevant in urban ecosystems (Goddard *et al.* 2010). Larger habitat patches sustain larger and more stable populations of birds (Evans *et al.,* 2009), amphibians (Parris, 2006), mammals (Magle, 2009), and carabid beetles (Sadler *et al.,* 2006). The importance of habitat connectivity has also been demonstrated in the urban landscape. The isolation of parks from other urban green spaces causes shifts in the faunal species assemblages favouring more generalist species (urban exploiters and urban adaptors) and exotics, while specialist species and other area sensitive species (urban avoiders) fade out (Nielsen *et al.*, 2014).

Most of the above studies concentrate solely on the above-ground communities of organisms such as plants, arthropods, gastropods and birds, while the impact of landscape characteristics on belowground communities has been scarcely investigated (Milano *et al.,* 2018). However, although limited, some studies have been uncovering the effects of landscape heterogeneity in different soil communities. Earthworm assemblages appear to be significantly affected by the percentage of impervious and green space surface, the amount of landscape cover types, patch density and landscape fragmentation (Xie *et al.*, 2018). For prokaryotes, total fungi, and arbuscular mycorrhizal fungi, distance to the city centre, urbanized land cover, and road density was associated with slightly homogenized communities (Liu et *al.,* 2022). In the case of collembolans and mites, patch isolation showed a significant negative effect on their abundance, and increased impervious surface cover alone did not affect collembolans, but mites showed a significant positive trend with increasing impervious surfaces (Piano *et al.*, 2020).

Therefore, land-use management, whether focused on local or landscape factors, significantly impacts soil and its functional role in maintaining ecosystem processes (Tresch *et al.,* 2019; Pantaloni *et al.,* 2022). Improvements in soil management to avoid a reduction in soil fauna diversity and to guarantee suitable soil conditions for soil organisms to develop are the only way to ensure long-term soil functioning and the provider of ecosystem services (Ertiban, 2019).

1.5. Objectives

The main purpose of this project was to assess the impact of urbanization in the abundance, diversity, and functions of soil fauna, to infer on soil ecology processes and quality across a range of urban land uses. Specific objectives were: i) to integrate information from various faunal groups and analyse if the factor land-use type affected the soil communities and which environmental variables are related with, and could act as

drivers for higher abundance, diversity, and the magnitude of functions of soil organisms; ii) to assess the relationship between diversity (taxonomic and functional) and the magnitude of a range of functions in urban ecosystems and iii) to advance the ecological theory in the soil component in urban environments, contributing to a better knowledge frame to inform on city management and planning decisions.

2. Materials and methods

2.1. Sampling and data collection

2.1.1 Selection of sampling sites

This study was conducted in the Municipality of Braga, with an area of 183.4 km^2 and a population of 192.494 inhabitants. It is located in the northern region of mainland Portugal, within the Cávado Subregion (NUT III), and is administratively part of the district of Braga, along with 13 other municipalities, whilst also serving as the district capital (Câmara Municipal de Braga, n.d.). Urban sites are characterized by their heterogeneity, presenting a diverse mixture of land uses. To be able to capture a representative selection of these different green spaces in the city, five categories of land use were sampled: unused land, lawns, flower beds, forests and roundabouts/roadsides (Figure 3). Five sites of each land use (25 sites in total) were selected to provide sample independence whilst representing the diverse range of land uses. Unused land consisted of abandoned meadows such as vacant lots, left in their natural state and not managed or cultivated, consisting of ground cover, shrubs, or even bare soil. Lawns were managed areas of grass, with scattered trees, serving as spaces for recreation and outdoor activities. These areas were subject to regular mowing and were assumed to receive additional care such as watering, fertilizing, and weed control. Flower beds were areas typically delineated by borders and specifically cultivated for growing ornamental plants and flowers, requiring regular maintenance, including watering, weeding, and fertilizing. Forests areas were characterized by high tree density, forming a forested landscape. Roundabouts/roadsides refer to the grassy verges, shrubs, or other spontaneous vegetation bordering roads, highways, or streets (Figure 3).



Figure 3. Illustrative photographs of selected categories of green space land-use types across the Municipality of Braga: (A) unused land; (B) lawn; (C) flower beds; (D) forest and (E) roundabouts/roadsides (photographs by the author).

In each category of land use, the selection of sites was made taking into account the variety of spatial scales, incorporating both larger and smaller sites. To accomplish this, green spaces were mapped through QGIS (Quantum GIS) using the satellite imagery from Google Earth (Figure 4). The area of each site was also calculated using these tools.

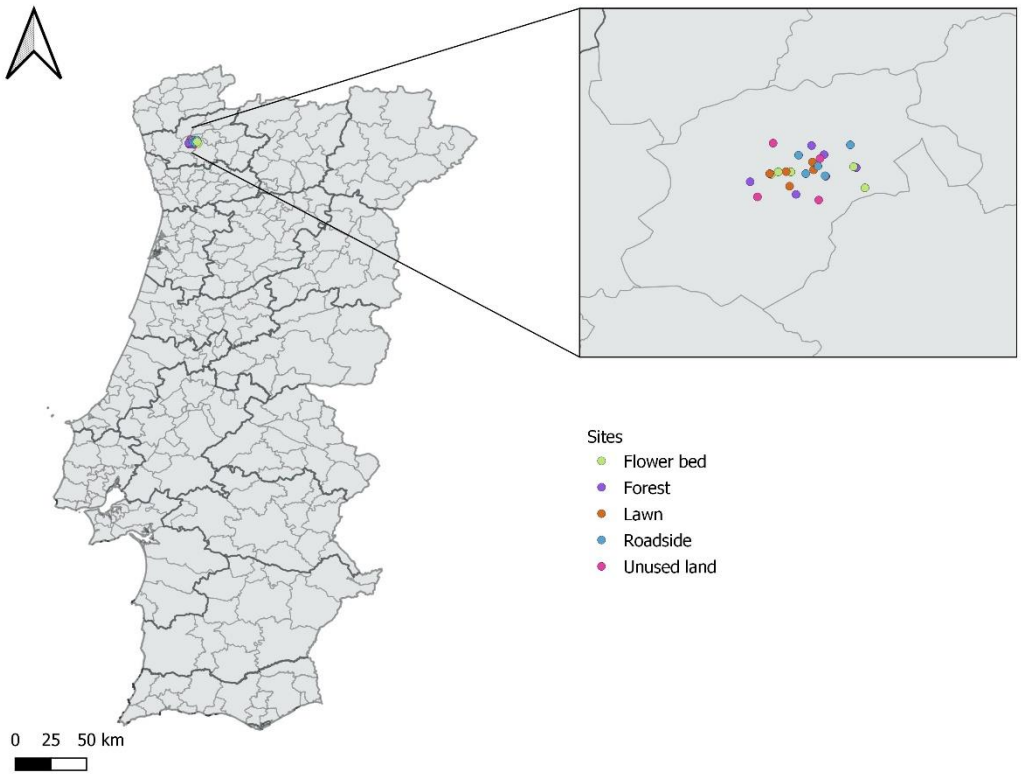


Figure 4. Selected sites representing different types of land use in the Municipality of Braga.

2.1.2. Sample Collection

All sites were sampled within seven days in the autumn, from the 22nd to the 28th November 2023. In each site, a composite soil sample composed of 9 subsamples at 15 m intervals in a grid-like pattern was taken (Figure 5). However, in some sites, such as flower beds and roadsides, it was not possible to follow this exact design, requiring occasional adaptations. Soil was collected down to 15 cm depth, the most biologically active zone (van den Hoogen *et al.* 2019), by extracting a 5 cm diameter soil core using a knife. The superficial litter layer and the first 5 cm of depth were stored separately for the mite and collembolan analyses. The remaining 10 cm of depth were stored for the nematode analyses and physical-chemical

parameters: conductivity, moisture, organic matter and pH. The sub-samples were carefully and thoroughly mixed, and any plant material (roots, leaves, twigs, moss, grasses, etc.), large rocks, and sizable animals (larger than a thumbnail) were removed. All samples were labelled and refrigerated (4° C) until processed.

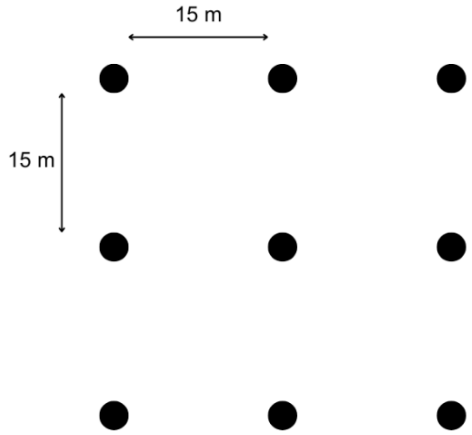


Figure 5. Soil sampling design, illustrating a grid-like pattern of sampling points with a 15-meter spacing between them in both horizontal and vertical directions.

2.2. Assessment of Physical-Chemical Parameters

From each sample, ca. 50 g of soil were passed through a 2 mm sieve prior to physical-chemical analyses. To determine soil moisture, 30 g of sieved soil was placed into a ventilated drying oven set to 105 °C until constant mass was achieved, for approximately 48-72 hours. Samples were allowed to cool down in a desiccator for approximately 30 min and then were weighted. The water content of soil was determined as the weight difference before and after drying (Wilke, 2005).

The organic matter content was determined by loss on ignition as described by Howard and Howard (1990). The previously dried soil samples were placed in a muffle furnace, where the organic matter was incinerated at 550 °C for 5 hours. After cooling in a desiccator for approximately 30 min, samples were weighted. The organic matter content was measured by the difference between the sample weight before and after incineration (Dean, 1974).

For the determination of pH and electrical conductivity, 6 g of soil samples, dried at ambient temperature, were mixed with 30 ml of distilled water (1:5 dilution), allowed to equilibrate for at least an hour, and then pH and electrical conductivity was measured using a pH electrode (WTW Multi 3320

multiparameter) (Walworth, 2011). Care was taken to measure first the conductivity, followed by the pH, to prevent any changes due to salinity in the pH electrode buffer solution.

2.3. Assessments of biodiversity and function

2.3.1. Plant cover

To evaluate the plant cover of each site, the quadrat sampling method was used. For this technique a square frame (50x50 cm) was randomly throw 3 times in each site. Then the proportion of different plant groups (legumes, grasses, forbs) and their respective percentage cover (percentage of ground covered by each functional plant group), and percentage of exposed soil was evaluated visually (Goodall, 1952).

2.3.2. Soil microfauna

Using the tray method, an adaptation of the Baermann Funnel (Whitehead & Hemming 1965), nematodes were extracted from 100 ml of soil from each sample. The soil was homogeneously distributed in a plastic tray with a net and a sheet of paper tissue. The edges of the tissue were then folded over the soil, and the tray was filled with water until the soil was soaked. During 72 hours the trays stood on a surface without agitation, enabling nematodes to migrate through the tissue into the tray. After the 72 hours, the net with the soil was carefully removed and the resulting suspension was poured through a 20 μm sieve to concentrate nematodes in a smaller volume of 10-20 ml. The observations of nematodes were conducted using an inverted microscope, and a simplified key (Ferris, 2011) was used to identify in trophic groups through the observation of mouth and upper digestive tract (Figure 6) (Yeates *et al.*, 1993), to family or up to genus level to be classified in functional groups (Bongers, 1990). The identification was done in nematode-counting plates, and only the first 100-200 observed nematodes were classified, followed by the quantification of the total number of nematodes in suspension.

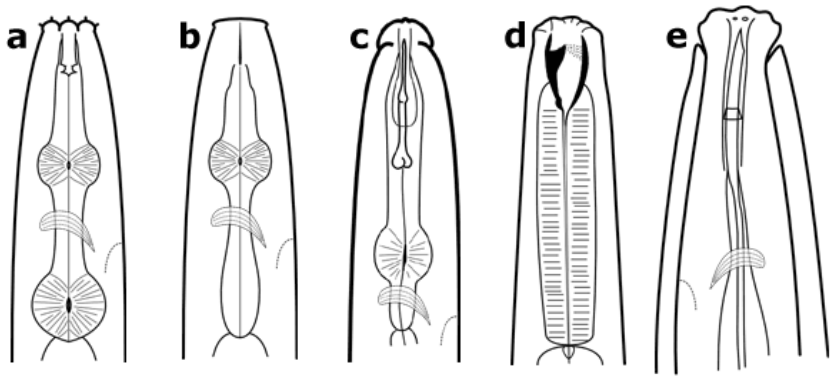


Figure 6. Nematode trophic groups based on the observation of mouth and upper digestive tract. (a) bacterivores, (b) fungivores, (c) herbivores, (d) predators and (e) omnivores. Figure credit: Ed Zaborski, University of Illinois.

The data collected were analysed through the NINJA software: Nematode Indicator Joint Analysis (Sieriebriennikov *et al.*, 2014), enabling the calculation of indices that provide bioindicators for disturbance of the soil environment and condition of the soil food web (Ferris *et al.*, 2001) (Table 1) and metabolic footprints, that provide metrics for the magnitudes of ecosystem functions and services (Ferris 2010, Sánchez-Moreno and Ferris, 2018). The soil microbial community activity was not measured directly but rather assessed by proxy based on the decomposition activity on the bacterial and fungal metabolic footprints obtained from the nematode community (Table 2).

Table 1. Description of nematode ecological indices: maturity, enrichment, structure, basal, and channel indices.

Index	Description	Reference
Maturity index	Weighted mean cp value of all nematodes in a given sample, informs on the overall successional condition of the soil community.	Bongers, 1990
Enrichment index	Measures the proportion of opportunistic bacterial and fungal feeders that respond quickly to the input of C and N sources.	
Structure index	Based on the relative abundance of nematodes in higher trophic groups and cp levels and indicates soil food web length and connectance.	Ferris *et al.,* 2001
Basal Index	Indicator of the prevalence of nematodes that are tolerant to stress (cp 2).	
Channel Index	Calculated as the proportional abundance of (cp 2) fungal feeders to enrichment opportunists; reflects the fungal or bacterial decomposition channel in the soil.	

Table 2. Description of enrichment, structure, bacterivore, fungivore, herbivore, omnivore, and predator metabolic footprints (according to Ferris, 2010).

Index	Description
Enrichment footprint	Metabolic footprint of those nematodes most rapidly responsive to resource enrichment, being an indicator of decomposition/mineralization of nutrients.
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 and food web complexity.
Bacterivore footprint	Metabolic footprint of bacterial-feeding nematodes, indicator of carbon and energy flow through the bacterial decomposition channel.
Fungivore footprint	Metabolic footprint of fungal-feeding nematodes, indicator of carbon and energy flow through the fungal decomposition channel.
Herbivore footprint	Metabolic footprint of herbivorous nematodes, indicator of carbon and energy flow through herbivory (primary production channel).
Omnivore footprint	Metabolic footprint of omnivorous nematodes, indicator of regulation.
Predator footprint	Metabolic footprint of predatory nematodes, indicator of regulation.

In addition to nematode ecological indices, the Shannon-Wiener, Pielou, and Margalef indices of the nematode communities were calculated using R software (version 4.4.2), with the package "vegan".

2.3.3. Soil mesofauna

To extract mites and collembolans, 200 ml of each sample were placed in a Macfadyen Extractor for 8 days. Soil samples were heated from above via hot air (45 °C) and cooled and slightly moistened by cool humidified air from below. Mites and collembolans moved towards the cool area and were collected through a funnel into the collecting vessels (containing 96 % ethanol) on the underside of the extractor (Macfadyen, 1961).

Extracted organisms were identified as mites or collembolans, following a key for the identification of groups of microarthropods (Srivastava, 2009) and quantified. Then, the Acari/Collembola index was calculated based on the densities of different groups. In natural conditions, this ratio is higher than one and tends to decline in more disturbed soils (Bachelier, 1986). In addition, collembola adaptation to the soil was assessed according to the eco-morphological index (EMI) (Parisi, 2001; Parisi *et al.*, 2005), which is based on observation of five easily identifiable morphological traits in each specimen: presence or absence of ocelli and setae (and/or scales), pigmentation, antenna length, and furcula length (Reis *et al.*, 2016). This index categorises collembola according to their degree of adaptation to the soil. Organisms with reduced antenna size, absence of ocelli and reduced pigmentation are assumed to be more adapted to the edaphic environment and consequently with less dispersion power (Figure 7). The higher the soil quality, the higher will be the number of microarthropod groups well-adapted to soil habitats (Parisi *et al.*, 2005).

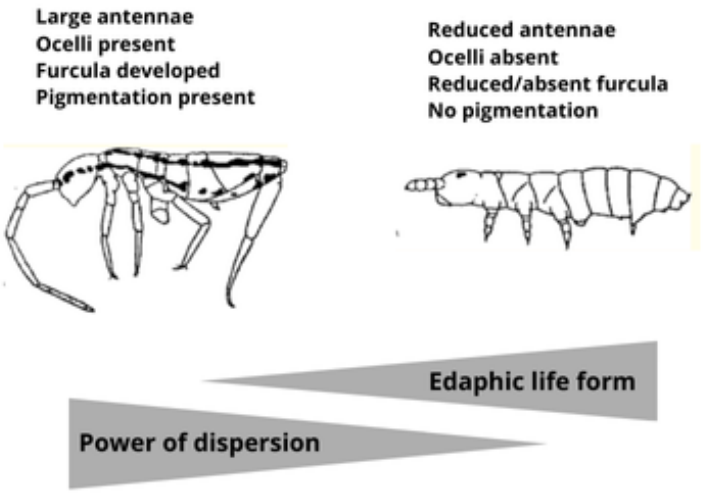


Figure 7. Representative diagram of the characteristics associated with a soil-dwelling collembola and a collembola with high dispersal ability. Adapted from Carvalho, 2012.

A partial EMI was assigned to each one these traits (Table 3), and their sum (Total EMI) indicated higher or lower adaptation to the soil by the collembola community. The higher the total EMI, the higher the adaptation to the soil and the lower the power of dispersion of the organisms, and vice versa. This index can vary from 0 to 20 (Oliveira Filho *et al.*, 2016). To each value of EMI was assigned a morphotype: collembolans

with EMI equal to or greater than 14 were considered edaphic organisms; between 12 and 8 were considered hemi(semi)-edaphic organisms; between 2 and 8 were considered epigeal organisms (Santos, 2008).

Table 3. Characteristics and values of the characteristics used in the calculation of the eco-morphological index (EMI) value and for distinguishing different morphotypes (adapted from Santos, 2008 & Carvalho, 2012).

Characteristic		Partial EMI Value
Ocelli	Present	0
	Absent	4
Antenna size	Antenna length > body length	0
	Antenna length > 0.5 x body length	2
	Antenna length < 0.5 x body length	4
Furcula	Present	0
	Present, but reduced	2
	Absent	4
Hairs/Scales	Present	0
	Absent	4
Pigmentation	Present and patterned	0
	Present, without patterns	2
	Absent	4

The mean trait value per community (mT) of collembola was calculated for each site. This mean considers the total number of collembola of a certain morphotype, divided by the abundance of all specimens and weighted by the specific value assigned to the traits relative to their form of life (EMI) (Vandewalle *et al.*, 2010). In other words, a site with a low mT indicates low adaptation to soil dwelling, whereas a site with a high mT suggests high adaptation (Santos *et al.,* 2018). The proportion of edaphic species in relation to the total was also calculated.

Considering each morphotype as a species, the Shannon-Wiener, Pielou, and Margalef indices of Collembola were calculated using R software (version 4.4.2), with the package "vegan". As mites were only assessed as a group and not subcategorised in functional groups, their diversity could not be analysed.

2.3.4. Soil macrofauna

Earthworm sampling was carried out by digging and hand sorting in the field. In each site a standard sampling effort of 1 person.hour actively searching for earthworms was performed. Specimens were collected in a plastic container filled with 96 % ethanol in the field, and immediately brought to the laboratory, where they were washed with 70 % ethanol to remove adhering soil. They were then stored in absolute ethanol, that was replaced ca. 2 weeks later.

The specimens were sorted into juvenile and adult forms and morphologically identified to species level, by earthworm expert Dr. Marie Luise Carolina Bartz (Centro Municipal de Cultura e Desenvolvimento de Idanha-a-Nova and Centre for Functional Ecology, University of Coimbra). Earthworm weight per sample was then determined in the laboratory. The identification conducted by the earthworm expert were made following an established identification key (Sherlock, 2012). This was based on external characters of the earthworms, including the head shape, the position of the clitellum, the arrangement of setae along the body, the position of the male pore, the position and form of the *tubercula pubertatis*, as well as specimen length and diameter (Figure 8).

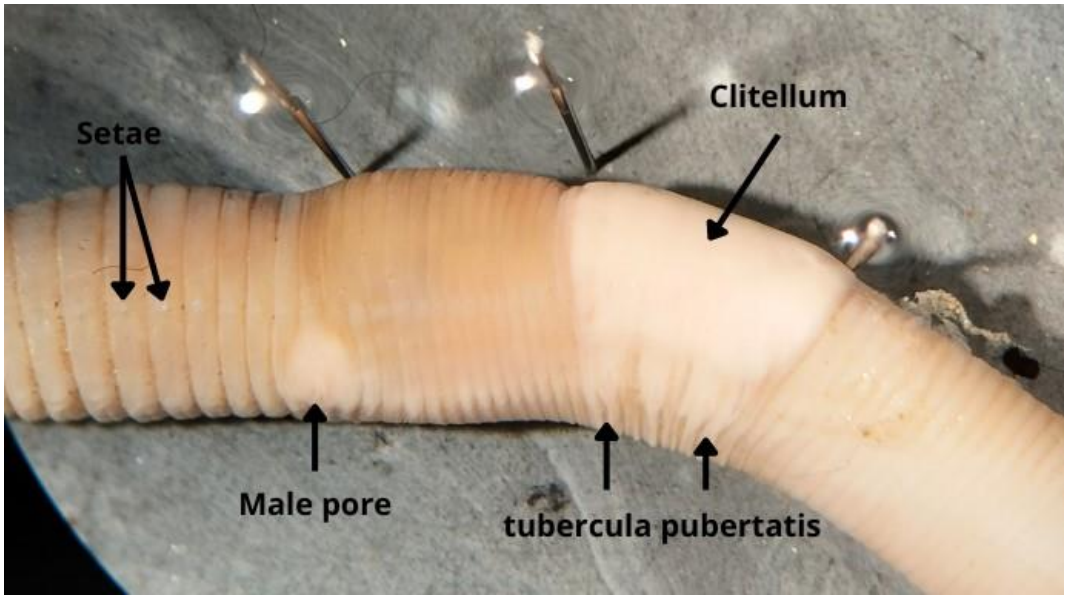


Figure 8. External earthworm features used for identification (photographed by the author using a stereomicroscope).

The Shannon-Wiener, Pielou and Margalef indices of the earthworm communities were calculated using R software (version 4.4.2), with the package “vegan”.

2.4. Data Integration

The influence of land-use type (flower beds, forests, lawns, roadsides and unused lands) on the ecological variables were determined by Generalized Linear Models using sing R software (version 4.4.2), with assumed normal distribution and identity link function, using glm() from the package “stats”. After fitting the Generalized Linear Model, a post hoc Least Significant Difference test, function LSD.test() from the package “agricolae” was conducted to identify which specific land-use type exhibited significant differences in their impact on the ecological variables. To gain a comprehensive understanding of the relationships among the different ecological indicators (collembolans, earthworms and nematodes), bivariate correlation tests were conducted, specifically Pearson correlation coefficient, function cor() from the package “stats”. This statistical method allowed to examine the strength and direction of the relationships between diverse ecological variables of the different groups of organisms.

All collected data was then normalized using RSTUDIO software, to ensure comparability and equitable contribution during the analysis. The Environmental variables (% Exposed Soil, % Forbs, % Grasses, % Legumes, Moisture, Organic Matter, Area, Electrical Conductivity and pH) were transformed using “scale” and the Ecological variables (% Acari/Colembolla, % Edaphic Collembola, Biomass of Earthworms and Nematodes, mT, Nematode Ecological Indices, Nematode Metabolic Footprints, Richness and Abundance of Earthworms, Nematodes and Collembola, Shannon, Pielou and Margalef indices of Earthworms, Nematodes and Collembola) were transformed using “log”.

To analyse possible co-correlated environmental variables, in R software (version 4.4.2), the Variance Inflation Factor (VIF), function vif() from the package “car” (Fox & Weisberg, 2019) was used. Variables were considered highly correlated if the VIF value was larger than 5. The % grasses was thus eliminated, and all variables then presented a VIF <5, making them acceptable for the canonical correspondence analysis (CCA). The CCA is a multivariate ordination method used to elucidate the relationships between biological assemblages of species and their environment (Braak & Verdonschot, 1995). The CCA was performed to determine the influence of the environmental variables on the Ecological Variables, and, separately, the influence of the environmental variables on Nematode families, Earthworm species and Collembola morphotypes.

3. Results

3.1. Effect of Land Use on Soil Organisms

The influence of land-use type (flower beds, roadsides, forests, unused lands and lawns) on the abundance and diversity of Nematodes (Annex 1), Collembolans (Annex 2 and 3), Mites (Annex 4), and Earthworms (Annex 5) was assessed per faunal group, followed by the analysis of group-specific indices, as described below.

3.1.1. Effect of Land use on Nematodes

Nematode abundance was not influenced by land-use type (Figure 9A), though nematode biomass was significantly affected (Figure 9B). Nematode communities had the largest biomass in flower beds, which was significantly larger than that of roadsides, unused lands and forests (with the smallest biomass).

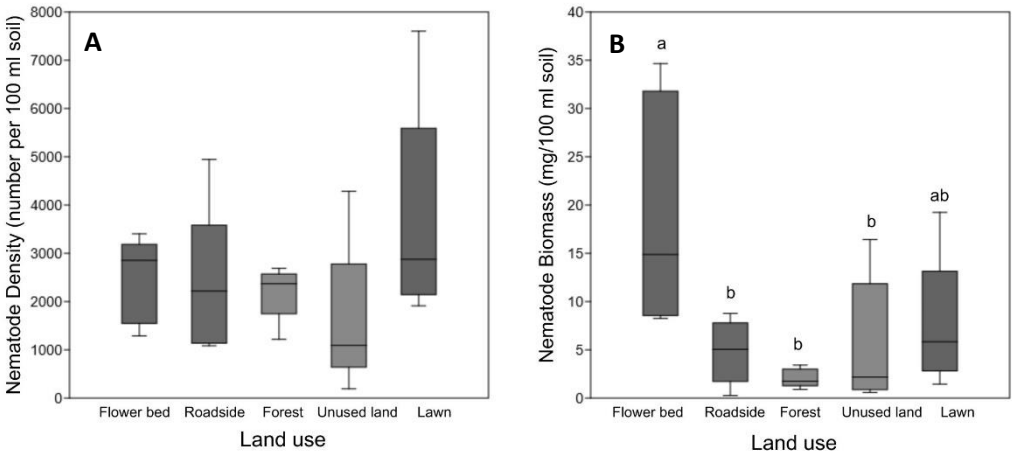


Figure 9. Nematode density (A) and biomass (B) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$).

Nematode diversity, evenness, and richness were significantly influenced by the land-use type. Nematode communities had the higher diversity in flower beds and forests, all significantly higher than that of lawns and roadsides (with the lower diversity) (Figure 10A). Evenness and richness had the highest values in flower beds, which were significantly different from those in lawns and roadsides (with the lower evenness

and richness). Unused lands and forests followed flowers beds in nematode community eveness and richness (Figure 10B and 10C).

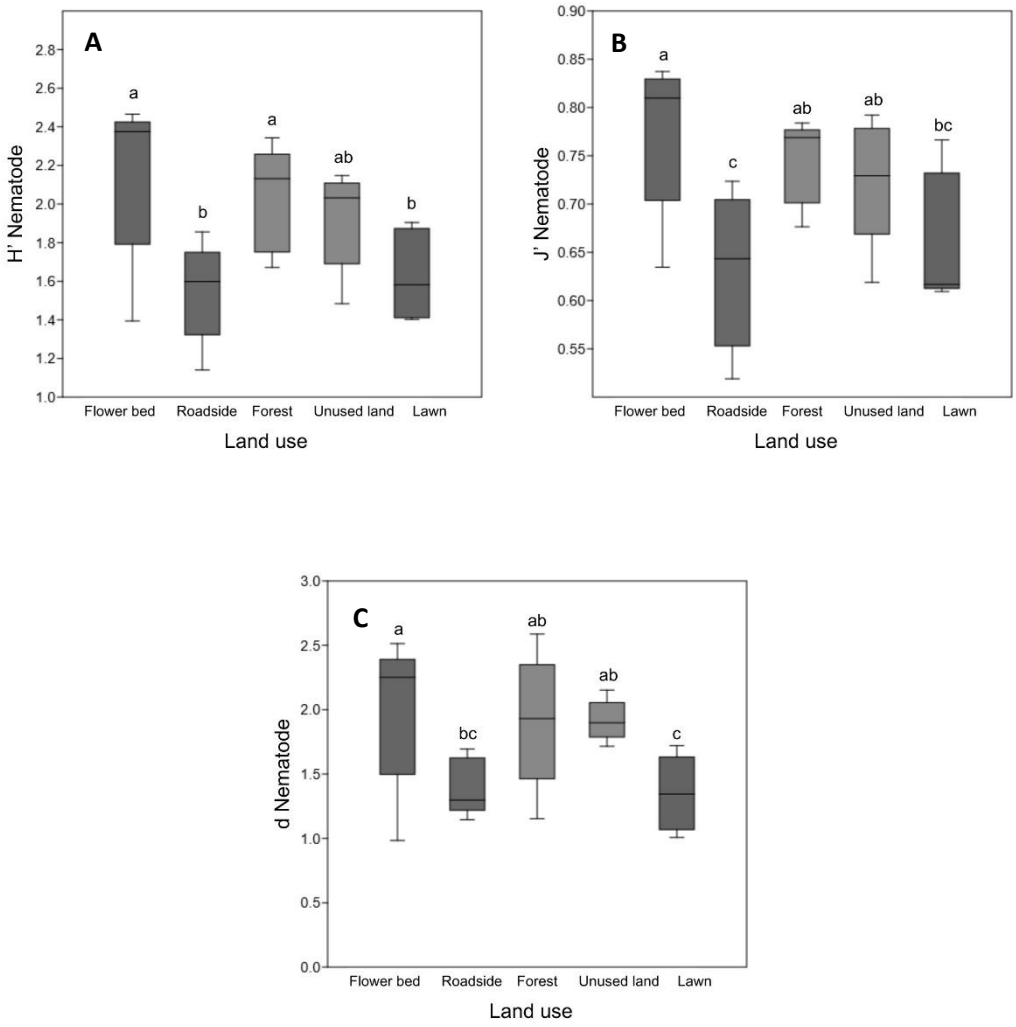
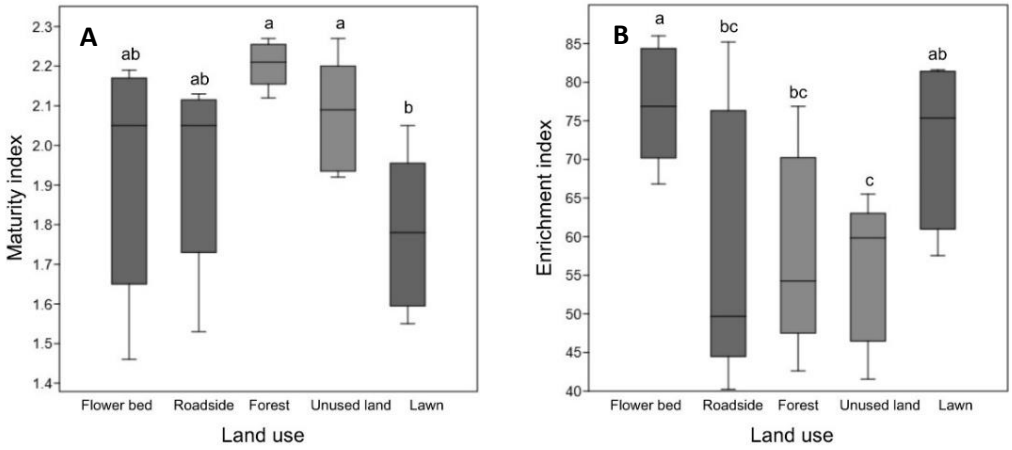


Figure 10. Nematode taxa diversity (Shannon index) (A); evenness (Pielou index) (B) and richness (Margalef index) (C) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$).

The nematode maturity index, enrichment index, structure index, basal index, and channel index were all influenced by land-use type. The maturity index was significantly higher in forests and unused lands than in lawns (with the lower maturity index), indicating a more stable and mature successional stage in the former sites (Figure 11A). The enrichment index had the highest values in flower beds, which was significantly

higher than that of forests, roadsides, and unused lands, followed by lawns with enrichment index higher than unused lands (with the lower enrichment index), indicating that flower beds and lawns are sites with high nutrient availability, and a strong opportunistic response to nutrient input (Figure 11B). For the structure index, nematode communities had the highest values in forests and surprisingly also in flower beds, both significantly higher than that of lawns and roadsides (with the lower structure indices) (Figure 11C), indicating that forests and flower beds had a higher proportion of persister nematodes in higher trophic groups, suggestive of longer food chains and well-connected soil food web. Nematode communities had the highest basal index in roadsides and unused lands, indicative of a simplified soil food web dominated by stress-tolerant nematodes (cp 2), which were significantly higher than that of flower beds (with the lower basal index) (Figure 11D). Finally, the channel index had the higher value in forests, roadsides, and unused lands, indicating a dominance of fungal decomposition pathways and more stable organic matter decomposition, all of which were significantly higher than flower beds (with the lower channel index), where bacterial decomposition was putatively dominant (Figure 11E).



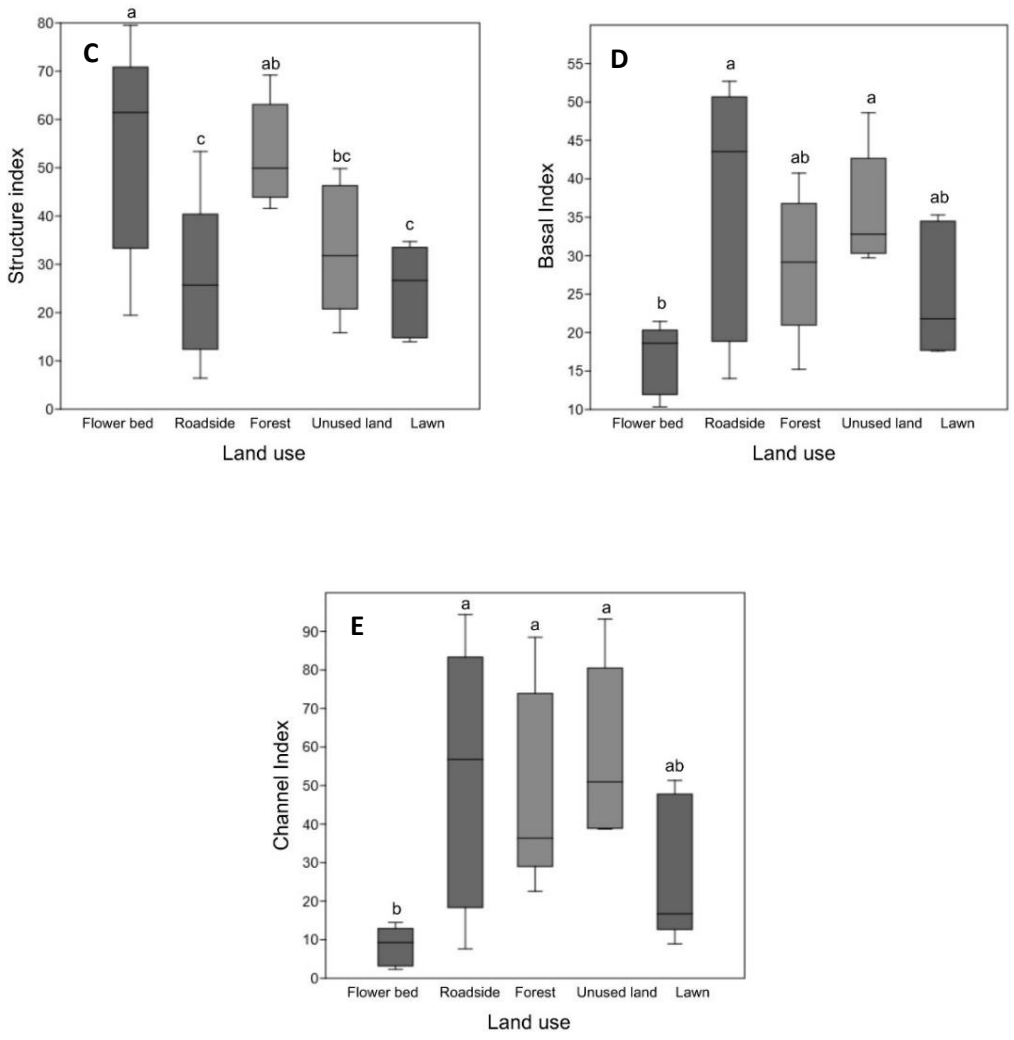


Figure 11. Nematode Maturity index (A); Enrichment index (B); Structure index (C); Basal index (D) and Channel index (E) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$).

There was a clear distinction in ecological conditions between the various land use types (Figure 12). According to the interpretation of faunal profiles by Ferris et al. (2001), both the forests and surprisingly flower beds, classified within Quadrat B, had low to moderate disturbance levels. These areas were characterized by nitrogen enrichment, decomposition channels with a balanced bacterial and fungal decomposition, a low C:N ratio and a maturing food web condition, capable of pest and disease regulation. Flower beds were overall more nutrient-enriched than forests, but with comparable food web structure. In

contrast, unused Lands, roadsides, and lawns, which classified within Quadrat A, represent highly disturbed sites. These areas exhibit nitrogen enrichment, bacterial-dominated decomposition channels, a low C:N ratio, and a disturbed food web condition, somewhat conducive to pests and diseases.

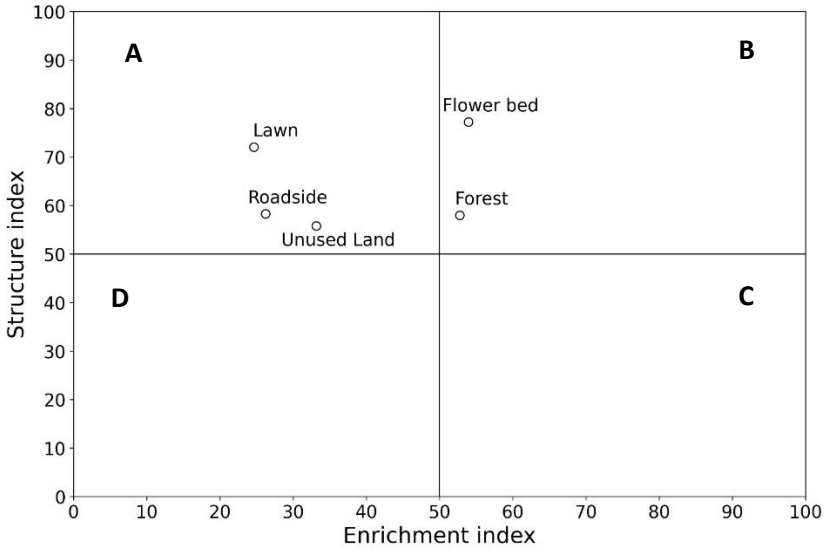


Figure 12. Faunal profile representing the structure and enrichment conditions of the soil food web for the different land use types sampled in the city of Braga. A, B, C and D represent the different quadrats.

The structure footprint remained unaffected by the different land-use types, although the enrichment footprint tended to be (p=0.09362) (Figure 13). The lack of significant variation in the structure footprint implies that metabolic activity of higher trophic levels of the soil food web was relatively stable regardless of land use, so natural regulation potential was comparable across land-use types. Additionally, the tendency of the enrichment footprint was higher in lawns, flower beds and roadsides and lower in forests and unused lands. However, within each land use, the relationship between the enrichment footprint and the structure footprint varied. Enrichment footprints were larger than structure footprints in lawns, flower beds and roadsides. Forests and unused lands, on the other hand, presented a more balanced structure: enrichment footprint, with the structure footprint slightly higher than the enrichment footprint.

Land-use type significantly influenced the footprints of herbivores, fungivores, and predators, albeit with only a marginal effect on the predator footprint (p=0.05143). In contrast, no significant influences were observed on the bacterivore and omnivore footprints (Figure 13). Flower beds had the highest herbivore footprint, significantly exceeding that of other land uses, suggesting a more active primary production channel (and correspondingly stronger herbivory pressure) compared to the other land uses. Also, for this land-use

type and for unused lands, the herbivore footprint was higher than bacterivore, fungivore and omnivore footprints, supporting the idea of a greater allocation of nutrients to the herbivory pressure rather than to decomposition. In contrast, forests had larger bacterivore, fungivore and omnivore footprints than herbivore footprints, suggesting a greater allocation of nutrients to the decomposition process rather than herbivory pressure. Lawns exhibited the highest fungivore footprints, significantly higher than that of unused lands and flower beds (with the lowest values). However, despite the high fungivore footprint in lawns, the bacterivore footprint was much higher, showing that in lawns the bacterivore decomposition channel was dominant, which was also evident for flower beds and roadsides, typical of disturbed soils with high nutrient availability. Both forests and unused lands had more balanced fungivore and bacterivore footprints. Forests and lawns had the highest predator footprints, significantly exceeding those of roadsides and unused lands (with the lower values).

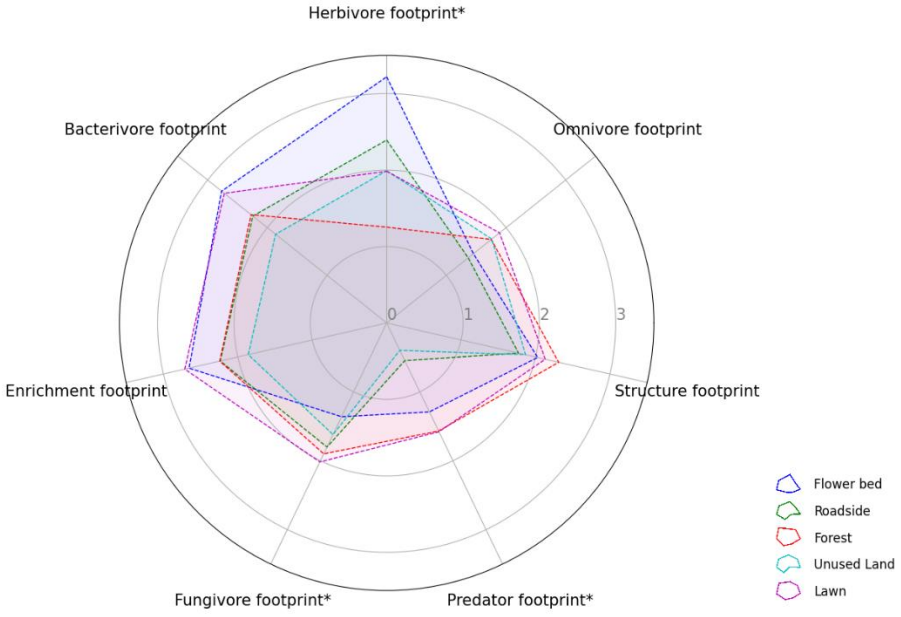


Figure 13. Nematode metabolic footprints as indicators of the magnitude of the ecosystem services performed by each nematode functional group sampled in the city of Braga for different land uses. Values are log-transformed means of five replicates per land-use type (flower bed, forest, Lawn, Roadside, Unused Land). Asterisk denotes statistically significant differences among land uses for a given footprint.

3.1.2. Effect of Land use on Collembolans and Mites

The abundance of collembolans was marginally affected (p= 0.05924) by land-use type (Figure 14A). Forests had the highest abundance of collembolans, with unused lands and lawns presenting the lower values. The abundance of mite communities was also affected by land-use type, with the forests having the highest abundance of all land uses (Figure 14B).

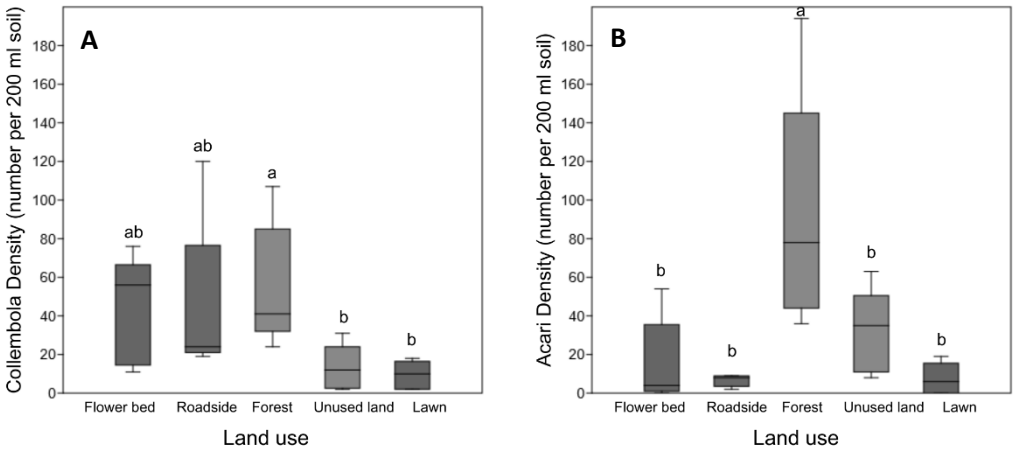


Figure 14. Collembolan density (A) and mite density (B) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$).

Unexpectedly, the diversity, evenness, and richness of Collembola were unaffected by land-use type (Figure 15). These indices were not calculated for mite communities, as the data includes only abundance information.

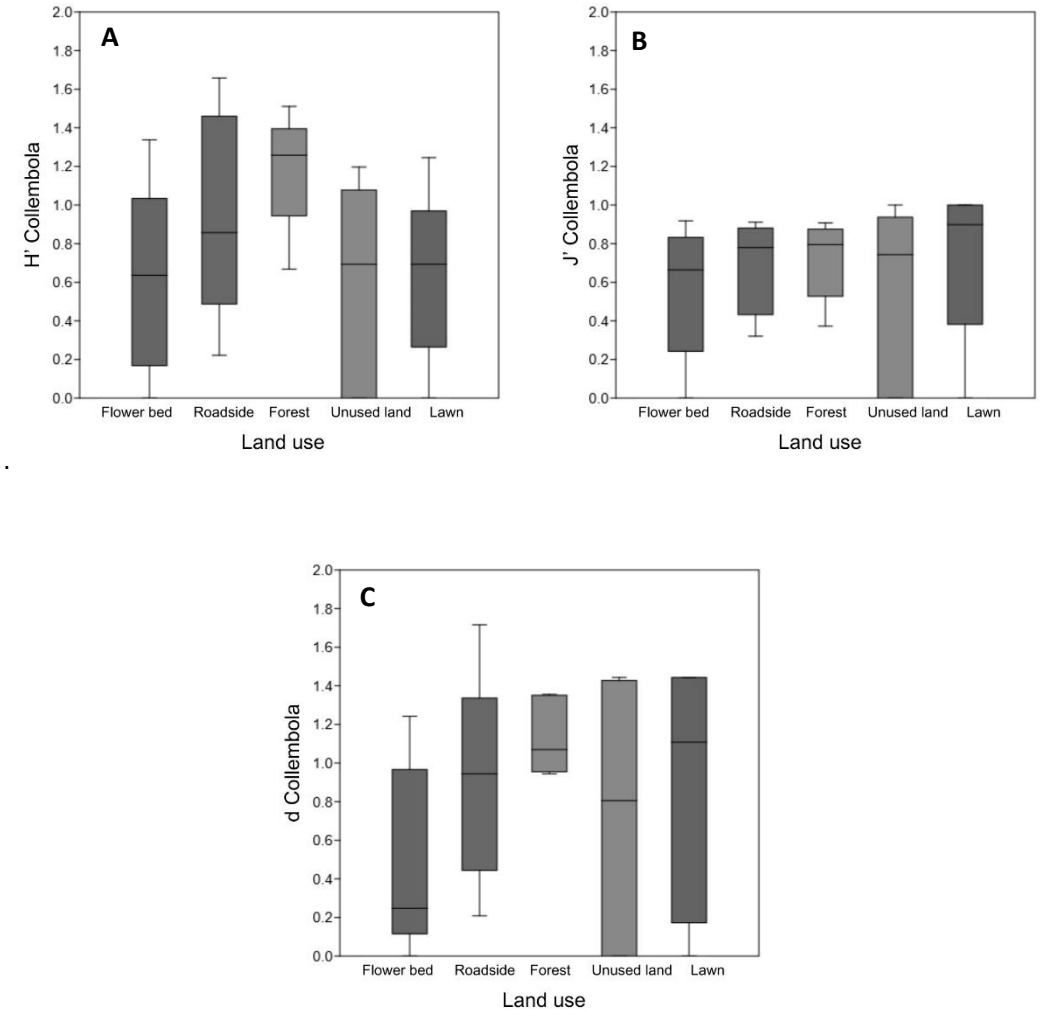


Figure 15. Collembola morphotype diversity (Shannon index) (A); evenness (Pielou index) (B) and richness (Margalef index) (C) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type.

No significant influence of land use was observed on the percentage of edaphic collembolans (Figure 16A). However, the mT (marginally significant; p= 0.05407) and the percentage of Acari/Collembola was significantly influenced by land-use type (Figure 16B and 16C). The mT values were significantly lower in roadsides than in all other land uses. Mite and collembolan communities had a higher Acari/Collembola ratio in unused lands, which was significantly higher than that of flower beds, lawns, forests and roadsides, with roadsides showing an exceptionally low value, indicative of a more disturbed environment, as the ratio decreases with increasing disturbance.

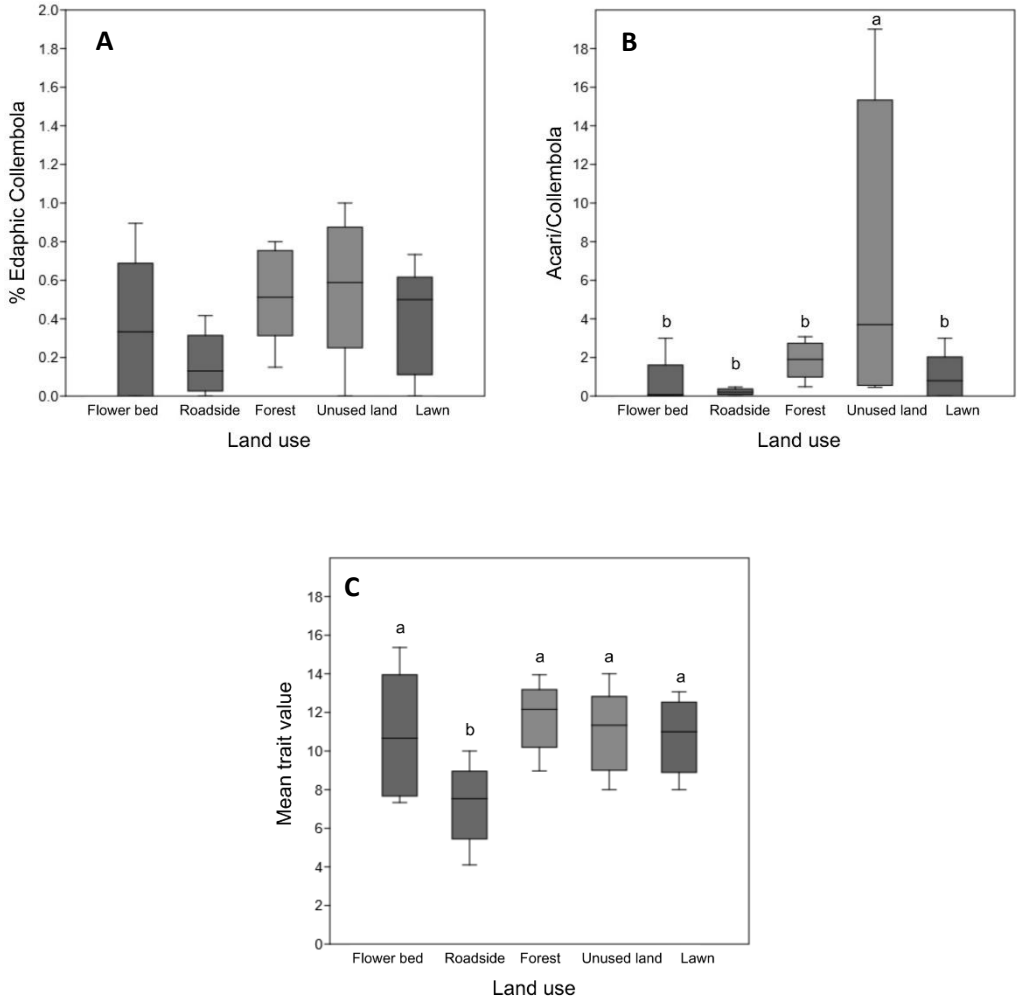


Figure 16. Edaphic Collembola (A); Acari/Collembola (B) and mean trait value (C) obtained from soil samples of different land use types in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$).

3.1.3. Effect of Land use on Earthworms

Earthworm abundance (Figure 17A) was significantly influenced by land-use type, whereas their combined biomass (Figure 17B) was not affected. Earthworm communities had the largest abundance in roadsides and lawns, which was significantly larger than that of forests and flower beds (with the smallest abundance).

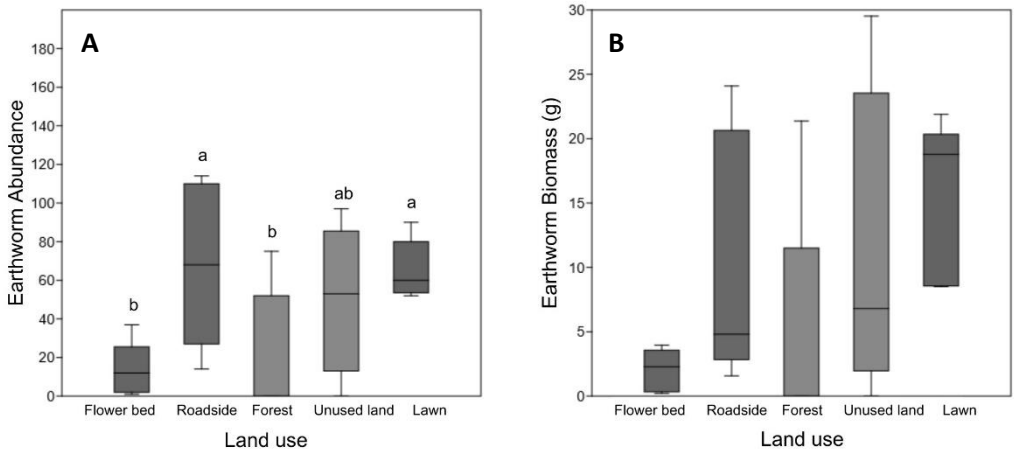
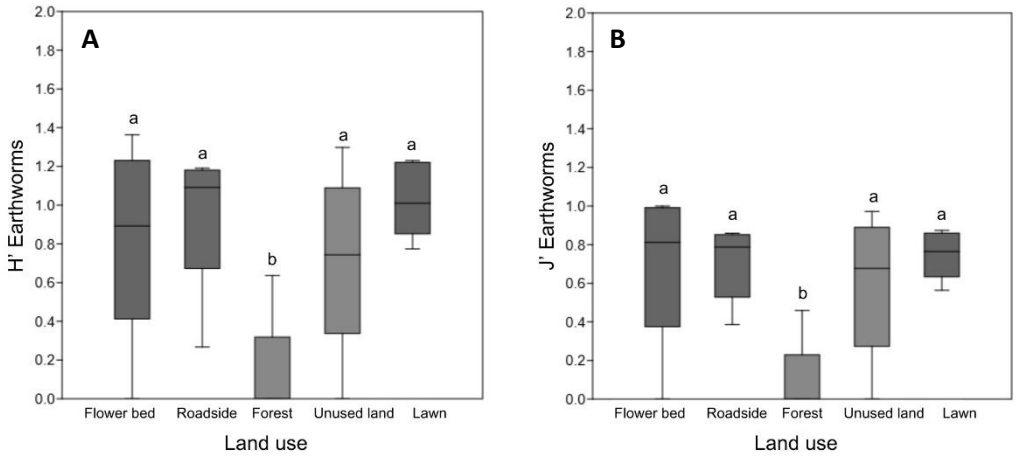


Figure 17. Earthworm species abundance (A) and biomass (B) obtained from active searches in different land use types sampled in the city of Braga. Box plots represent median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$).

Earthworm diversity and evenness but not richness differed across land-use types (Figure 18). Earthworm communities in lawns, roadsides, flower beds, and unused lands had the highest diversity and evenness, all significantly higher than that of forests (with the lower diversity and evenness) (Figure 18A and 18B). This suggests that lawns, roadsides, flower beds, and unused lands had a greater species diversity and a more balanced distribution of individuals across species, while forests had fewer species and might be dominated by a few species.



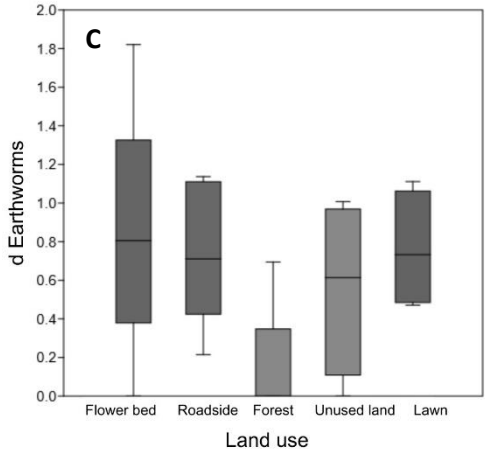


Figure 18. Earthworm species diversity (Shannon index) (A); evenness (Pielou index) (B) and richness (Margalef index) (C) obtained from active searches in different land use types sampled in the city of Braga. Values are median, quartiles and maximal/minimal values of five samples per land-use type. Boxes followed by the same letter are not significantly different according to GLM, LSD ($p<0.05$).

3.2. Relationships between faunal groups

Metrics calculated from the different communities of faunal groups in soil were correlated, varying in the same or opposite directions (Annex 6). Nematode biomass was found to be positively correlated with earthworm richness (r: 0.440; p: 0.028) and negatively correlated with Collembola richness (r: -0.453; p: 0.023) and diversity (r: -0.456; p: 0.022). Nematode diversity (r: -0.469; p: 0.018) and evenness (r: -0.486; p: 0.014) were negatively correlated with earthworm abundance, and nematode richness was positively correlated with the mT of Collembola (r: 0.412; p: 0.041). Interestingly, Collembola diversity was negatively correlated with earthworm diversity (r: -0.433; p: 0.031).

The herbivory footprint was positively correlated with the earthworm richness (r: 0.436; p: 0.029) and negatively correlated with Collembola richness (r: -0.445; p: 0.026) and diversity (r: -0.427; p: 0.033). The maturity index was positively correlated with Collembola abundance (r: 0.402; p: 0.046) and unexpectedly, the nematode structure index was negatively correlated with earthworm abundance (r: -0.465; p: 0.019).

3.3. Influence of the environmental variables on soil organisms

The influence of environmental variables (% Exposed Soil, % Forbs, % Legumes, Moisture, Organic Matter, Area, Electrical Conductivity and pH) (Annex 7) on soil organisms was analysed and visualized using a CCA biplot. The environmental variable, % grasses, was not included in the CCA analyses since the VIF value indicated a possible co-correlation with the other environmental variables.

3.3.1. Influence of the environmental variables on faunal Abundance and Biomass

For the nematodes, earthworms and collembolans Abundance and Biomass, the environmental variables explained 67% of the variance in the data. Axis 1 explained 53 % of the total variance, which was significant (p=0.001), while Axis 2 explained 10 % of the total variance, also significant (p=0.005). Axis 1 divided faunal communities mostly according to the influence of organic matter (-0.82), percentage of legumes in the vegetation (-0.41), and moisture (-0.21) (on the negative side), and the influence of pH (0.36) and exposed soil (0.19) (on the positive side). Axis 2 divided faunal communities with electrical conductivity (0.43) and site area (-0,50). Faunal communities were then markedly differentiated, with earthworms positioned in the positive side of axis 1 and negative side of axis 2; Nematode biomass positioned in both positive side of axis 1 and 2; Collembola and nematode abundance positioned in the negative side of axis 1 and positive side of axis 2 and mites abundance positioned in both negative side of axis 1 and 2 (Figure 19A). Distinctions in environmental variables and faunal communities could be based on land use, as 95% ellipsoids of roadsides and lawns appear to be more restricted, and that of unused lands appear to encompass all the other land uses (Figure 19B).

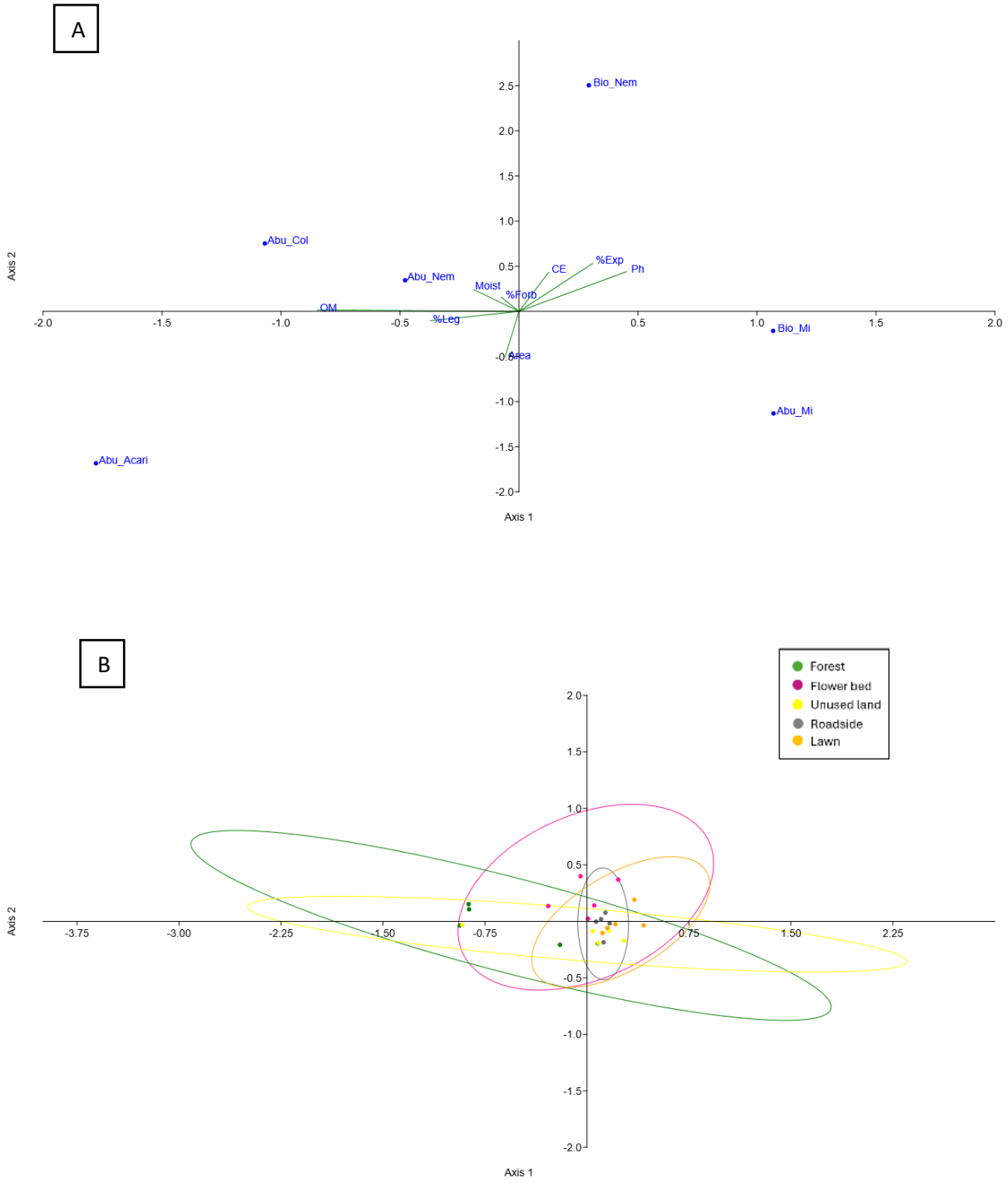


Figure 19. Canonical correspondence analysis of the influence of the environmental variables on nematodes, earthworms and collembolans Richness, Abundance and Biomass. Eigenvalues were 0.08451and 0.01639 for the first and the second axis, respectively. (A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and the nematodes, earthworms and collembolans Richness, Abundance and Biomass (Triplot

amplitude: x1). (B) overlapping of 95% ellipses for the land use groups. Riq_Nem: nematode richness; Abu_Nem: nematode abundance; Bio_Nem: nematode biomass; Riq_Mi: earthworm richness; Abu_Mi: earthworm abundance; Bio_Mi: earthworm biomass; Riq_Col: Collembola richness; Abu_Col: Collembola abundance; Abu_Acari: mites abundance; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.

3.3.2. Influence of the environmental variables on faunal biodiversity indices

Environmental variables accounted for 56% of the variation in biodiversity indices for nematodes, earthworms, and collembolans. Axis 1 explained 48% of the variance, which was significant (p=0.01), while Axis 2 explained 6%, but this was not significant (p=0.313). Axis 1 mainly differentiated biodiversity indices based on two sets of opposing influences. On the negative side, pH (-0.68) and percentage of exposed soil (-0.20). On the positive side, organic matter (0.68), percentage of legumes cover in the vegetation (0.36), and site area (0.20). The faunal biodiversity indices showed a clear distinction, with collembolans and nematode diversity, evenness, and richness grouped on the positive side, while earthworm diversity, evenness, and richness were positioned on the negative side (Figure 20). No clear distinctions in faunal biodiversity indices based on land use were identified, as 95% ellipsoids for datapoints in each group are mostly overlapped, with several common datapoints (Annex 8).

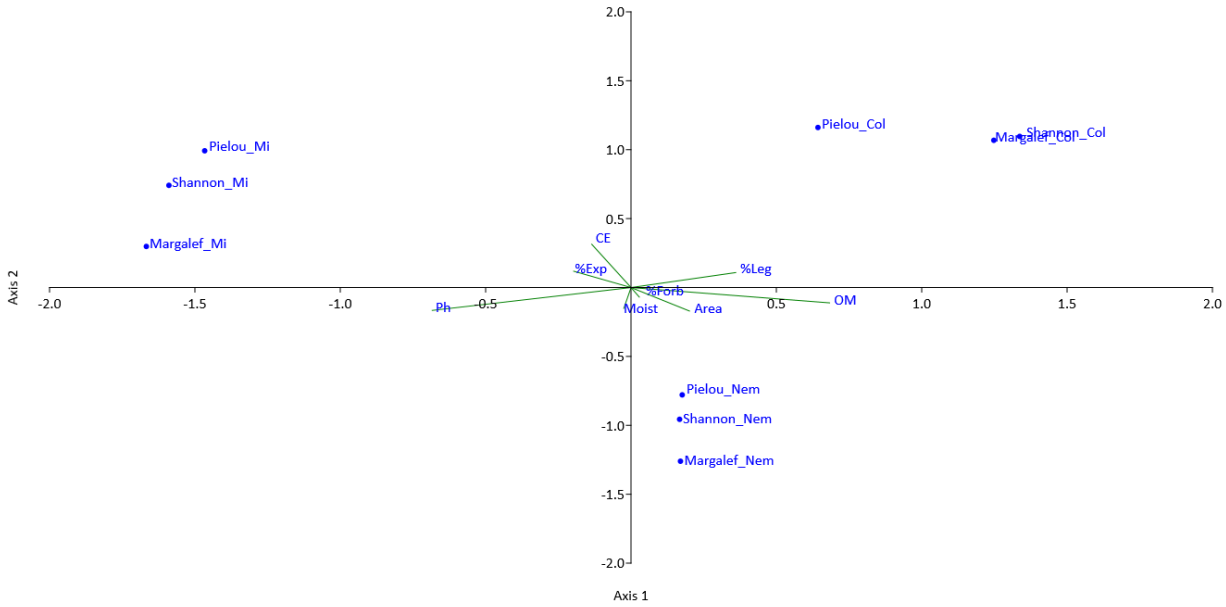


Figure 20. Canonical correspondence analysis of the influence of the environmental variables on nematodes, earthworms and collembolans biodiversity indices. Eigenvalues were 0.08516 and 0.01082 for the first and the second axis, respectively. Canonical correspondence analysis bi-plot representing the environmental variable vectors and the nematodes, earthworms and collembolans Shannon, Pielou and Margalef indices (Triplot amplitude: x1). Shannon_Nem: nematode Shannon index; Pielou_Nem: nematode Pielou index; Margalef_Nem: nematode Margalef index; Shannon_Mi: earthworm Shannon index; Pielou_Mi: earthworm Pielou index; Margalef_Mi: earthworm Margalef index; Shannon_Col: Collembola Shannon index; Pielou_Col: Collembola Pielou index; Margalef_Col: Collembola Margalef index; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.

3.3.3. Influence of the environmental variables on nematode community indices

Environmental variables explained 63% of the variance in nematode community indices. Axis 1, which accounted for 55% of the variance was statistically significant (p=0.007). Axis 2, which explained 7% of the variance, was not significant (p=0.13). On Axis 1, the nematode community indices were mostly influenced by moisture (-0.66), electrical conductivity (-0.54), percentage of exposed soil (-0.44), and percentage of forbs cover in the vegetation (-0.22) and pH (-0.18). On the positive side, legume cover (0.45) and site area (0.17) were the dominant factors. The nematode community indices were clearly differentiated, with the channel index and basal index on the positive side, and the structure index and enrichment index on the negative side. However, the maturity index did not appear to be influenced by the environment variables present, as it

was located closer to the origin of axis 1 (Figure 21). No clear distinctions in faunal communities based on land use were identified, as 95% ellipsoids for datapoints in each group are mostly overlapped, with several common datapoints (Annex 9).

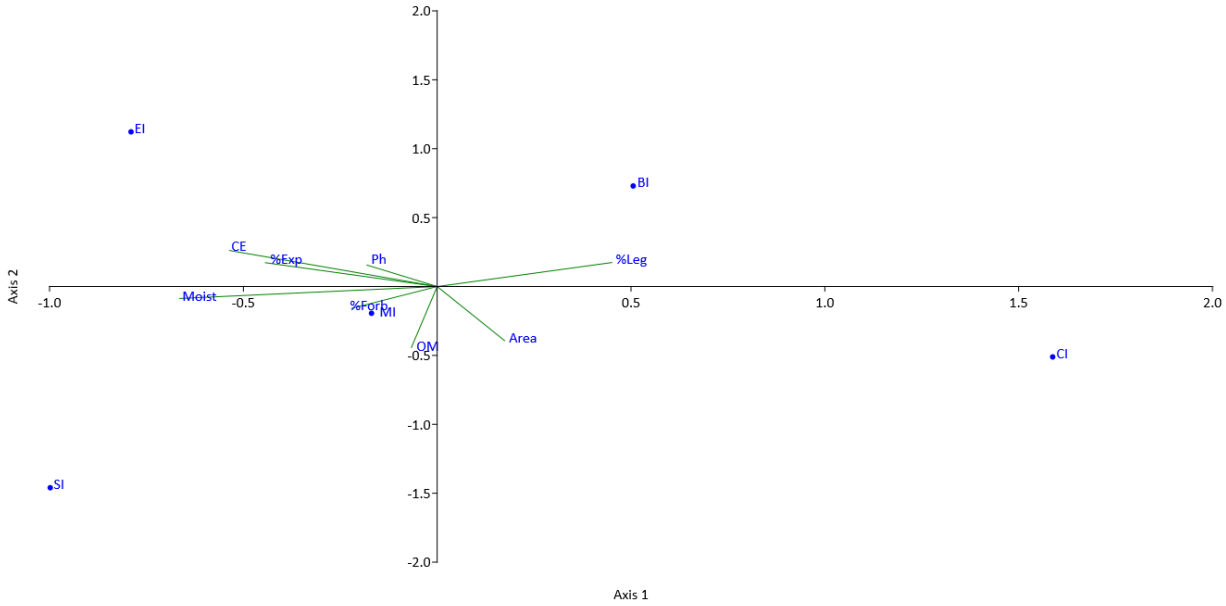


Figure 21. Canonical correspondence analysis of the influence of the environmental variables on nematode community indices. Eigenvalues were 0.0121 and 0.001593 for the first and the second axis, respectively. Canonical correspondence analysis bi-plot representing the environmental variable vectors and the Maturity, Enrichment, Structure, Basal, and Channel indices (Triplot amplitude: x1). MI: Maturity index; EI: Enrichment index; SI: Structure index; BI: Basal index; CI: Channel index; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.

3.3.4. Influence of the environmental variables on nematode metabolic footprints

For the nematode metabolic footprints, a meaningful relationship with the variables analysed could not be inferred (Annex 10A). Additionally, there was also no apparent clear distinction in metabolic footprints distribution according to land use (Annex 10B).

3.3.5. Influence of the environmental variables on Collembolans and Mites parameters

Regarding the Collembola parameters, there was no significant relationship between mT, the percentage of edaphic collembolans, and the percentage of Acari/Collembola with the environmental

variables analysed (Annex 11A). Furthermore, the distribution of Collembola parameters showed no clear distinction based on land use (Annex 11B).

3.3.6. Influence of the environmental variables on nematode taxonomical diversity

The environmental variables analysed explained 44% of the variance in the taxonomical composition of nematode communities. Axis 1 accounted for 13% of the total variance, which was statistically significant (p=0.028), while Axis 2 explained 10% of the variance, also significant (p=0.006). Negative contributors on Axis 1 included pH (-0.29), percentage of exposed soil (-0.25), electrical conductivity (-0.19) and the percentage of legume cover in the vegetation (-0.15). In contrast, positive influences were associated with site area (0.77), organic matter (0.65), moisture (0.30), and the percentage of forbs cover in the vegetation (0.36). Axis 2 further distinguished nematode taxonomical diversity, highlighting negative effects from organic matter (-0.31) and legume cover percentage (-0.34). Positive contributions on Axis 2 included percentage of forb cover in the vegetation (0.54), pH (0.54), electrical conductivity (0.52), moisture (0.44) and percentage of exposed soil (0.25). The environmental variables showed to influence the distribution of nematode families, as evidenced by the clear separation of certain families along the positive and negative sides of axis 1 and axis 2. However, there does not appear to be a clear pattern related to trophic levels or cp values. Omnivores and, particularly, predators were mostly concentrated on the positive side of axis 1, with predators further clustered in the positive regions of both axes 1 and 2. Notably, plant-parasitic nematodes such as *Meloidogyne* and *Pratylenchus*, which are among the top 10 plant-parasitic nematodes (Jones *et al.,* 2013), showed distinct ordination patterns. *Meloidogyne* was influenced by environmental variables on the negative side of axis 1 and the positive side of axis 2, while *Pratylenchus* was influenced by variables on the negative side of axis 1, but appeared uninfluenced by axis 2 variables, as it was positioned near the origin of the axis. Some nematode families appeared to be only slightly influenced by the studied variables, and therefore their abundance may not be strongly affected by the considered environmental variables (Figure 22). No apparent clear distinction in data distribution according to land use was found, as 95% ellipsoids for datapoints in each group mostly overlapped with the others (Annex 12).

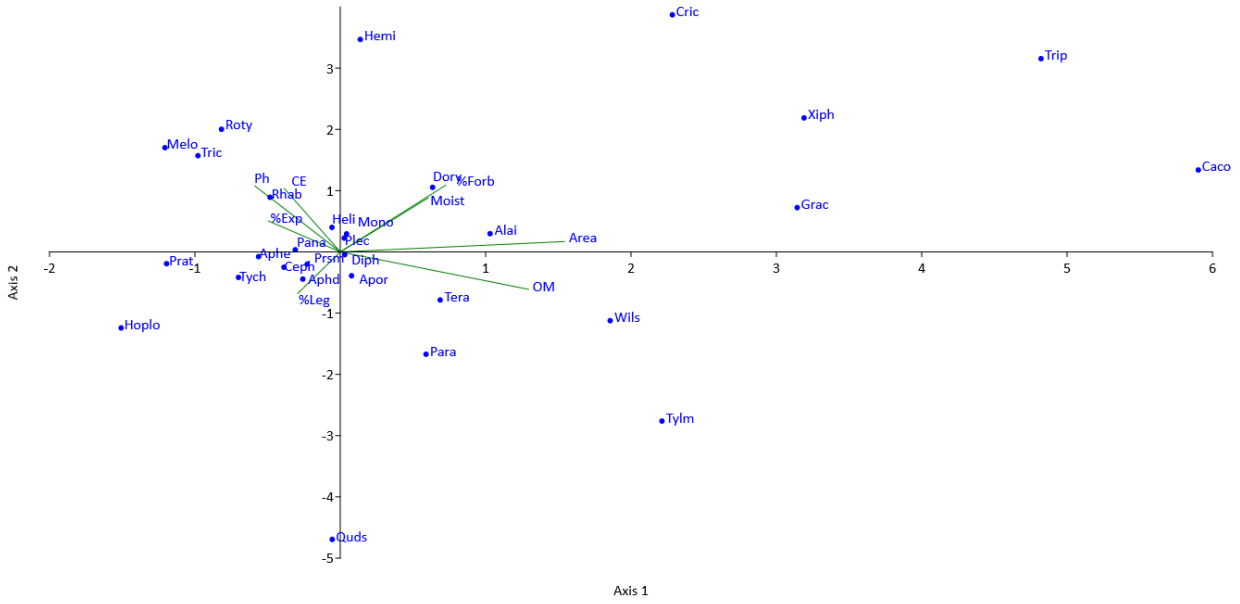


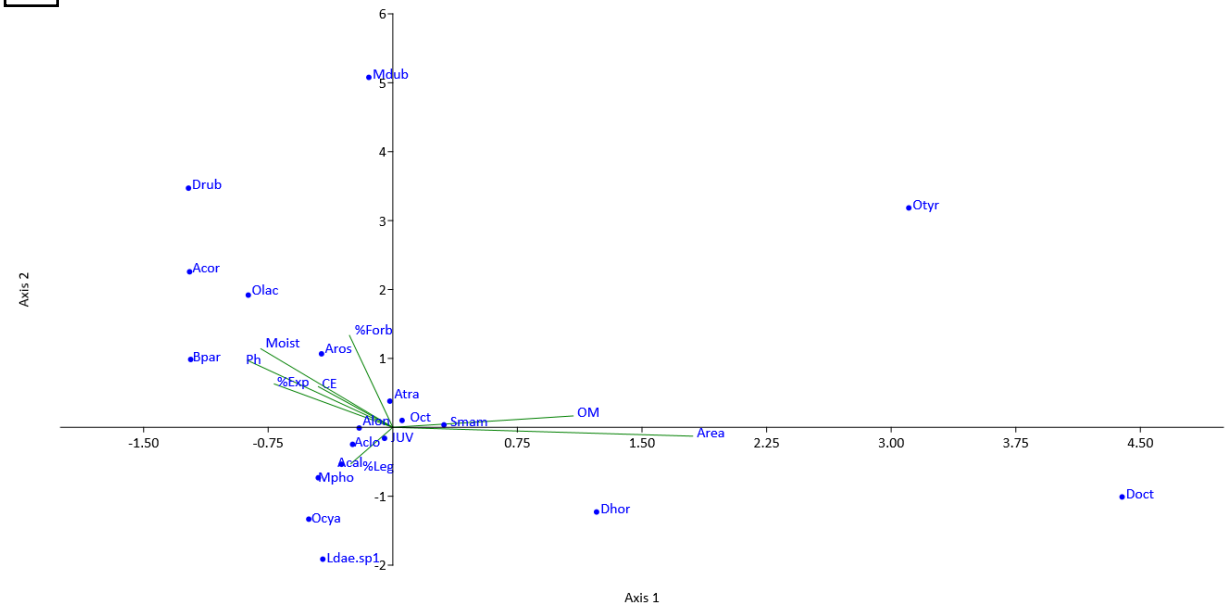
Figure 22. Canonical correspondence analysis of the influence of the environmental variables on nematode taxonomical diversity. Eigenvalues were 0.12 and 0.09364 for the first and the second axis, respectively. Canonical correspondence analysis bi-plot representing the environmental variable vectors and the nematode taxonomical diversity (Triplot amplitude: x2). Aphd: Aphelenchoididae; Aphe: Aphelenchidae; Diph: Diphtherophoridae; Tylm: *Tylencholaimus*; Pana: Panagrolaimidae; Ceph: Cephalobidae; Rhab: Rhabditidae; Tera: Teratocephalidae; Prsm: Prismatolaimidae; Wils: Wilsonema; Plec: Plectidae; Alai: Alaimidae; Heli: *Helicotylenchus*; Roty: *Rotylenchus*; Para: *Paratylenchus*; Prat: *Pratylenchus*; Melo: *Meloidogyne*; Tych: *Tylenchorhynchus*; Hoplo: *Hoplolaimus*; Xiph: *Xiphinema*; Grac: Gracilacus; Cric: *Criconematidae*; Caco: *Cacopaurus*; Hemi: *Hemicycliophora*; Tric: Trichodoridae; Quds: Qudsianematidae; Apor: Aporcelaimidae; Dory: Dorylaimidae; Mono: Mononchidae; Trip: Tripylidae; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.

3.3.7. Influence of the environmental variables on earthworm taxonomical diversity

For the earthworm taxonomical diversity, the environmental variables explained 43% of the variance in the data. Axis 1 explained 15 % of the total variance, which was significant (p=0.003), while Axis 2 explained 7% of the total variance, which was non-significant (p=0.453). Axis 1 divided earthworm species mostly according to the pH (-0.44), moisture (-0.40), percentage of exposed soil (-0.36), electric conductivity (-0.22) and percentage of forbs and legumes cover in the vegetation (-0.13; -0.12) (on the negative side), and the influence of area site (0.90) and organic matter (0.54) (on the positive side). The environmental variables

showed to influence the distribution of earthworm species, as evidenced by the clear separation of certain species along the positive and negative sides of axis 1. There was identified a known invasive earthworm species, the *Amynthas corticis*, positioned on the negative side of the axis. However, there were some species that appear to be only slightly influenced by the studied variables (Figure 23A). The forests appear to have a quite distinct species composition and environmental conditions, while the other types of land use show more similarity to each other, as 95% ellipsoids for datapoints in each group are mostly overlapped, with several common datapoints, except for forests (Figure 23B).

A



B

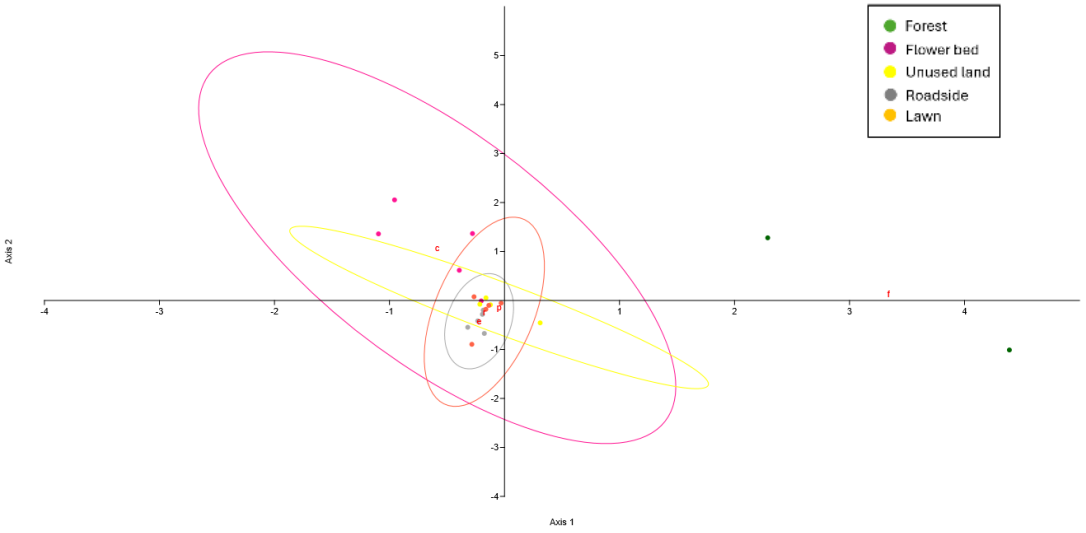


Figure 23. Canonical correspondence analysis of the influence of the environmental variables on earthworm taxonomical diversity. Eigenvalues were 0.7277 and 0.3439 for the first and the second axis, respectively. A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and earthworm taxonomical diversity (Triplot amplitude: x2). (B) overlapping of 95% ellipses for the land use groups. Drub: *Dendodrilus rubidus*; Bpar: *Bimastos parvus*; Otyr: *Octolasion tyrtaeum*; Ocya: *Octolasion cyaneum*; Olac: *Octolasion cyaneumlacteum*; Doct: *Dendrobaena octaedra*; Dhor: *Dendrobaena hortensis*; Drub: *Dendrobaena rubidus*; Smam: *Satchellius mammalis*; Aclo: *Aporrectodea chlorotica*; Acal: *Aporrectodea caliginosa*; Alon: *Aporrectodea longa*; Aros: *Aporrectodea rosea*; Atra: *Aporrectodea trapezoides*; Ldae sp1: *Lumbricidae*, Mdub: *Microscolex dubius*; Mpho: *Microscolex phosphoreus*; Acor: *Amynthas corticis*; Oct: *Octodrilus sp*; JUV: juveniles that cannot be identified at the family, genus, or species level; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.

3.3.8. Influence of the environmental variables on Collembola morphotypic diversity

No significant relationship was found between collembolans morphotypic diversity and the analysed variables (Annex 13A). Furthermore, there was no clear distinction in the distribution of collembolans morphotypic diversity based on land use (Annex 13B).

4. Discussion

4.1. Analysis of Soil Food Webs and Biological Soil Condition Across Land Uses

Soil conditions of the sampled sites in the Braga Municipality differed across land-use types and were poorer in human-impacted sites such as lawns and roadsides than in the unmanaged forest sites. Lawns and roadsides had nutrient-rich soils but basal food webs. The nutrient-rich soils of flower beds were dominated by plant-parasitic nematodes, as were the more ecologically complex unused lands. Forests, as expected, had the most balanced and stable soil food webs, representing the better condition of ecological status.

Flower beds stood out with the highest enrichment index, closely followed by lawns, revealing high nutrient availability and an opportunistic response to nutrient inputs in these environments. The enrichment footprint, although not statistically significant, also tended to be maximal in flower beds and lawns. This is unsurprising, given that these green spaces are actively managed, being irrigated and putatively receiving fertilizer inputs, that may substantially change soil characteristics, leading to high levels of nutrient-rich organic matter (Yu *et al.,* 2012). Bacterivores, as expected, prevailed in these nutrient-rich environments, with the bacterivore footprint being much higher than the fungivore footprint, typical of disturbed soils rich in nutrients (Ferris, 2010). Accordingly, a more active bacterial decomposition channel is associated with unstable and enriched conditions, whereas as the fungal decomposition channel is associated with more stable soil conditions (Costa, 2024). Human activities in such managed urban environments introduce additional nutrients (e.g. through fertilization) to promote plant growth, that indirectly increase substrates available for decomposers, thereby boosting the growth of microbial consumers (Li *et al.,* 2022; Gurr *et al.,* 2003; Neher, 2010; Yeates, 1999). In flower beds, lawns, and roadsides, the enrichment footprint (representing prey) was higher than the structure footprint (representing predators). Under stable environmental conditions, such an abundance of prey would support a high number of predators, however, putatively due to impacts in these soils, predator numbers remained low (Ferris, 2010; Li *et al.,* 2024). The frequent fertilization and tillage led to simplified food webs dominated by opportunistic taxa that can thrive under these conditions, and a low abundance of predatory nematodes (Siebert *et al.*, 2020; Bongers and Bongers 1998; Dmowska & Kozlowska 1988). In accordance with this, the food webs of these soils were found to be less mature (as indicated by a lower maturity index). However, unexpectedly, lawns (along with forests), showed the higher predator footprint. This high predation activity in lawns is unexpected, since predators, as persisters, are known to be sensitive to disturbances in soil (Zhao & Neher, 2013; Song *et al.,*

2019). This may be explained by the high bacterivore activity in these systems, representing abundant available prey. This is supported by the concept of amplifiable prey, species that are either neutral or beneficial to management objectives and whose abundance can be enhanced by provision of specific resources (Ferris *et al.,* 2012). However, even though lawns had more predators, their structure footprint was still lower than the enrichment footprint. This suggests that without disturbances, there would be even more predators in these soils. The lower predation activity was found in roadsides and is here attributed to putative high levels of heavy metal that are often detected in these areas (Pagotto *et al.,* 2001; Wang & Zhang, 2018) and have been reported to affect predatory nematodes (Georgieva *et al.,* 2022; Šalamún *et al.,* 2012; Pen-Mouratov *et al.,* 2008). However, heavy metal concentrations were not determined in this study.

The highest herbivore footprint was expectedly found in flower beds indicating a stronger herbivory pressure than in other land-use types. In fact, the herbivory footprint in flower beds exceeded the bacterivore, fungivore, and omnivore footprints, suggesting that more nutrients are allocated into the primary production than to the decomposition channel. Although I did not find specific studies regarding food web analyses in flower beds, intensively managed agroecosystems have been shown to have low densities of bacterial and fungal feeders, but thriving populations of plant feeders (Siebert *et al.,* 2020). Flower beds in Braga are regularly tended to by the city gardeners, with frequent replanting, inversion tillage, fertilization and weed control, which may hinder the establishment of predators since they are very sensitive to disturbances (Zhao & Neher, 2013; Song *et al.,* 2019), disrupting the regulation of herbivore populations, leading to communities governed by herbivory.

In contrast with managed soils, forests ecosystems have been overall reported as having structured and complex soil food webs (Ferris *et al.,* 2001; Zhang *et al.,* 2015; Duarte *et al.,* 2024). Our results are in agreement with previous findings, as forests had the highest maturity index, reflecting a more stable and mature successional stage. In Braga, these areas are generally scarcely managed, and not in close proximity to dense traffic or construction, possibly allowing the development of a more balanced, stable ecosystem (Yeates, 2007; Lišková *et al.,* 2008). In accordance, the predator footprint was higher in forest soils than in those of other land uses. Forests also presented the more balanced enrichment: structure footprint ratio of all land uses. This suggests that available nutrients are invested into creating long, complex food webs, which suggests that these systems are in metabolic balance (Ferris, 2010; Cardoso *et al.,* 2016). Soils in forests usually have high cellulosic and lignified organic matter (high C:N ratio) that fuels the fungal decomposition

channel (Ferris *et al.,* 2001; Neher *et al.,* 2005; Ruess, 2003). If the decomposition in the sampled forest soils was not dominated by Fungi, the proportion of fungal and bacterial decomposition was more balanced than that of managed sites. Both bacteria and fungi have distinct roles and complementary functions in soil ecosystems, and when they coexist, they amplify each other's effects (Deveau *et al.,* 2018). Forest soils also had the lower herbivore footprint of all land uses, and presented larger bacterivore, fungivore and omnivore footprints than herbivore footprints, suggesting a greater allocation of nutrients to the decomposition process rather than herbivory pressure. The low levels of herbivory pressure found in forests are possibly because, herbivorous nematodes prefer plant species with fine roots, which is not the case of more lignified roots typical of trees (Čerevková *et al.,* 2021). Interestingly, unused lands apparently presented an overall ecological state comparable to forests, with a similar maturity index, enrichment: structure footprint ratio and fungivore: bacterivore decomposition. Unused lands are increasingly recognized as a valuable ecological resource, by providing ecosystem services (Herrmann *et al.,* 2016; Nassauer & Raskin 2014) and supporting a high diversity of organisms (Delgado de la flor *et al.,* 2020; Perry et al., 2020). However, most of these unused lands were previously urban systems that used to have buildings or paved surfaces (e.g., residential homes, parking lots) (Green *et al.,* 2016). In fact, residual construction materials from the building and foundation, including bricks, stones, metals and wood, along with stripped paint are often left on site or buried in the soil, that can alter soil physical and chemical properties (Sharma *et al.,* 2015). This could explain why, contrary to forests, in unused lands the primary production channel was dominant over the decomposition channel, as other studies also reported a large abundance of plant-parasitic nematodes in unused lands (Sharma *et al.,* 2015). Taking these results together with the observed lower abundance of predators, it is likely that heavy metal contents can also affect soil food webs in these systems (Sharma *et al.,* 2015). Unused lands such as vacant lots in urban environments often have (and often had in our sites) leftover construction materials (Sharma *et al.,* 2015), which may be particularly noxious to predatory nematodes, that are very sensitive to disturbances (Zhao & Neher, 2013; Song *et al.,* 2019).

Interestingly, the metabolic footprint of bacterivores did not differ significantly across land-use types. The urbanization effect, with disturbances enhancing nutrient deposition, but also the deposition of pollutants (Biasioli *et al.*, 2006) and spreading resources uniformly across all green spaces, could foster bacterial growth in similar ways across land-use types (Thornton & Matlack, 2002). Bacterivorous nematodes are the dominant trophic group on a global scale (Van Den Hoogen *et al.,* 2019), while the other trophic groups are more responsive to environmental conditions (Zhao *et al.,* 2014; Čerevková, 2006; Háněl, 2001). However, as

discussed above, the relationship between fungal and bacterivore decomposition varied within each site, which allowed further conclusions. Fungivore footprints did vary, with lawns reaching the highest values among all land uses, followed by forests. The high lignin and cellulose content in these areas may help explain this phenomenon, as they both were the green spaces that supported more woody vegetation (Afzal *et al.,* 2023). Another possible explanation could be also associated with the trees present in these land uses, but specifically the leaf litter and the root biomass. This was reported in other studies, where high abundances of fungivore nematodes were found in forests (Keith *et al.,* 2009; Kitagami *et al.,* 2017; Duarte *et al.,* 2024) putatively due to the low pH that results from the soil acidifications by litter accumulation (Kitagami *et al.,* 2017), the large root biomass (Keith *et al.,* 2009) or because of the consumption of largely available mycorrhizal fungi (Cesarz *et al.,* 2015). In fact, in forests, there was a higher abundance of Aphelenchoididae (Fu2), followed by *Tylencholaimus* (Fu4), and since Fu4 nematodes are known to have feeding preferences for mycorrhizal fungi (Cesarz *et al.,* 2015), it can be inferred that this high fungivore footprint was likely due to a high abundance of mycorrhizal fungi in these forests, as also suggested for forests in the north of Portugal (Duarte *et al.,* 2024). Similar findings were also reported in lawns with grown trees compared to lawns with no trees, supported a greater abundance of fungi and fungal-feeding nematodes over bacteria and bacterial-feeding nematodes (Francini *et al.,* 2018). In fact, the samples of this study were taken in the autumn, a time with more accumulation of leaf litter, which could also explain this results, as reported in other studies (Duarte *et al.,* 2024). However, in this study, neither the trees, nor leaflitter nor root biomass were evaluated.

4.2. Effect of Land Use on Soil Communities

The land-use type influenced the community structure of all investigated soil organisms: nematodes, collembolans, mites and earthworms. However, the specific effects of each land use on the soil community structure varied among the different taxonomic groups. In fact, the same type of land-use can create favourable conditions for some organisms while being less optimal for others (Amossé *et al.,* 2016). Nematodes, collembolans and mites had more structured communities in forests, and earthworms had more structured communities in lawns and roadsides. This variation arises as different soil organisms are associated with different soil components, each responding uniquely to changes in land use (Szabó *et al.,* 2023).

Flower beds appear to be the most favourable habitat for nematode communities, with this group of organisms having the largest biomass, diversity, evenness and richness in these sites. However, this does

not necessarily imply flower bed soils had the most structured nematode communities – they were dominated by plant-parasitic nematodes. Taken together, plant-parasitic nematodes have a higher average biomass compared to other trophic groups (Ferris, 2018). These nematodes were also identified to genus level, whereas nematodes in other trophic groups were mostly classified to family level, which may have introduced some bias in the diversity analyses. The regular fertilization and irrigation in these areas likely boost populations of primary consumers, such as herbivores (Siebert *et al.,* 2020), whose high biomass and diverse families were actually dominated by *Meloidogyne*, *Pratylenchus* and *Helicotylenchus*, the first two being among the top 3 plant-parasitic nematodes worldwide (Jones *et al.,* 2013). In contrast, the high values for nematode diversity, evenness and richness, comparable to those found in flower beds, were matched to a more stable and complex nematode community, in forests (Yeates, 2007).

Unsurprisingly, forests emerged as the prime habitat for thriving collembolans and mite communities. Land-use type did not significantly affect morphotype diversity, evenness, richness, or the proportion of edaphic collembolans. The effect of soil management practices has been deemed responsible for the homogenisation of collembolan communities among different sites (Joimel *et al.,* 2017). However, land use did impact, although only marginally, collembolan and mite abundance and collembolan mean trait value. As expected, the abundance of both collembolans and mites was higher in forests, which was in accordance with other studies of urban forests (Yu *et al.,* 2021; Huang *et al.,* 2020). This could be explained by the least anthropogenic disturbance in forests (Machado *et al.,* 2019), and by the thicker litter layers providing food resources and favourable habitats (Petersen & Luxton; 1982; Huang *et al.,* 2020). Roadsides are often associated with high levels of heavy metals (Pagotto *et al.,* 2001; Wang & Zhang, 2018). Multiple studies have shown heavy metals to be harmful to soil fauna, and particularly affecting organisms like collembolans, which are highly susceptible to heavy metal toxicity (Crommentuijn *et al.,* 1993; Herbert *et al.,* 2004). It is therefore unsurprising that mean trait values of collembolans were notably lower along roadsides compared to other land uses, indicating larger populations of fast-dispersing, surface-dwelling (epigeous) species. Unlike edaphic species, which are less mobile and more sensitive to environmental stress, epigeous species can quickly colonize favourable conditions. Accordingly, mites were also scarce in roadside soils, which is in agreement with previous findings, that further attributed this effect to the stronger impacts of heavy metal concentration (Manu *et al.,* 2018; Manu *et al.,* 2017). Also, the Acari:Collembola ratio was exceptionally low in roadsides indicating this was the most disturbed environment among all land uses (Bachelier, 1986).

Given the above results for other groups of organisms, it was surprising to find earthworms were most abundant in roadsides and lawns, and scarcer in forests and flower beds. The lower abundance in forests is in line with the known habitat preferences of earthworms (Lavelle, 1983; Rutgers *et al.,* 2016). In flower beds, the regular use of chemical fertilizers and pesticides can be noxious to earthworms (Chauhan, 2014). Chemical fertilizers have been shown to reduce earthworm abundance indirectly, by reducing soil pH (Pahalvi *et al.,* 2021), whereas the application of broad-spectrum pesticides can be lethal to earthworms, even to those residing deep in the soil (Edwards & Bohlen, 1996). Additionally, tillage in flower beds may cause mechanical damage to earthworms or expose them to predation, including by birds (Nuutinen, 1992; Ahmed & Al-Mutairi, 2022). In fact, many researchers observed more earthworms in natural ecosystems than in ploughed agricultural systems (Edward & Bohlen, 1996; Kladivko, 2003), even though tillage is perhaps one of many contrasting factors in these systems. Interestingly, despite these disparities in abundance, earthworm biomass remained stable across land use types. This was not supported by previous studies that reported roadsides and lawns to hold larger biomass of earthworms (Raczuk & Pokora, 2008; Tóth *et al.,* 2020). The diversity and evenness of earthworm communities were similar across all land uses, except for forests, were they presented the lowest values. This is likely due to the often-thick uppermost organic layer composed of recalcitrant plant material, which supports few, if any, earthworms, aside from a few tolerant species (Addison, 2009). However, the number of species, or earthworm richness, was comparable across all land use types. This stability in species richness might be due to human activity facilitating the spread of earthworms, potentially leading to more similar communities across different urban green spaces (Tóth *et al.,* 2020). But, this might not apply to all earthworm species as, in this study, the invasive earthworms *Amynthas corticis* were found only in flower beds. Invasive ecosystem engineers are amongst the most troubling invasive species, since they have the ability to drastically modify their environments and affect the availability of resources for indigenous species (Crooks, 2002). *Amynthas corticis* (Kinberg, 1867) originated from Asia and is known for its aggressive, snake-like movements (Görres & Melnichuk, 2012) and elevated metabolic rates that contribute significantly to its invasive potential (Greiner *et al.,* 2012). Earthworms in the genus *Amynthas* have often been reported in wood-mulched ornamental beds, as these substrates appear to be an optimal habitat for this genus (Bellitürk *et al.,* 2015; Görres & Melnichuk, 2012). Additionally, earthworm cocoons can be dispersed between flower beds attached to gardening tools, equipment, soles of boots and shoes (Dupont *et al.*, 2012, Baumann *et al.,* 2024).

4.3. Influence of the environmental variables on soil organisms

4.3.1. Influence of the environmental variables on faunal biodiversity

Land use alone influenced soil communities, but environmental variables played an equally, if not more, important role in shaping community composition across land-use types. These environmental factors contributed to shared community patterns across different land uses, highlighting that soil communities are shaped by a complex interplay between land use and local environmental conditions (Kanianska *et al.,* 2016; Birkhofer *et al.,* 2012). Collembolans, mites and nematodes appeared to have preferences for the same type of environment variables (organic matter content, percentage of legumes in the vegetation, moisture and site area), while, interestingly, earthworms appeared to be most influenced by a completely different set of environmental variables (soil pH and percentage of exposed soil).

The multivariate ordination analyses constrained by soil parameters as environmental variables could not extricate differences according to land-use type in the soil communities. This suggests that environmental drivers play a significant role, and that these are not consistent in each land use. However, the 95% probability ellipses for the variance of soil communities in roadsides and lawns were more restrict than those of other land uses, suggesting that the communities in these areas have less variability in their responses to environmental conditions. This is probably due to uniform management practices, such as mowing, fertilization, and soil compaction, that limit habitat diversity (Costa, 2024). In contrast, the broader ellipses for unused lands and forests indicates greater ecological variability. These areas typically have a diverse mix of vegetation, varied soil conditions creating rich microhabitats (Hishi *et al.,* 2008; Bolger *et al.,* 2013). This complexity possibly allowed a wider range of organisms to thrive and respond differently to environmental changes.

Nematode community parameters, including abundance, diversity, evenness, and richness (but excluding biomass), showed a negative correlation with pH, a pattern also reported in other studies (Zhao *et al.,* 2017). Similarly, these parameters were negatively related with exposed soil. Although I could not find studies that linked directly the nematode communities and exposed soil, previous studies identified a positive relation between NDVI and nematode communities (Li *et al*., 2022). Nematode abundance was positively correlated with soil moisture, as nematodes live in water films surrounding soil particles and thus favour environments with sufficient humidity to support their movement and feeding (Brussaard, 1997; Costa *et al.,* 2011, Costa, 2024). Organic matter and the proportion of legumes in vegetation positively correlated with

nematode parameters, except for biomass. This likely occurs because increased organic matter supports microbial growth, which in turn boosts populations of microbial consumers, like microbivorous nematodes and enhances their abundance and diversity (Neilson *et al.,* 2020; Costa, 2024). As for legumes, it has been reported that legume addition and legume density were considerable drivers of total nematode abundance (Zhao *et al.,* 2023). This study also demonstrated that larger site areas support greater diversity, evenness, and richness in nematode communities. However, there is a lack of studies exploring the relationship between nematode communities and site area, making it difficult to validate this finding. Nematode biomass, interestingly, did not respond to the same environmental variables as the other nematode parameters. Instead, nematode biomass was related to the same environmental variables as earthworms, pH and exposed soil. This may be related to the larger biomass of herbivore nematodes, as previously presented (Ferris, 2018). Previous studies showed that lower soil pH seems to favour the populations of plant-parasitic nematodes (Melakeberhan *et al.,* 2004; Noronha *et al.,* 2020). I would further suggest that excessive plant parasitism by herbivore nematodes may be causing the soil to become exposed by reducing plant establishment and/or performance, a common finding in agroecosystems (Jones *et al.,* 2013).

Similarly to nematodes, collembolan community parameters (abundance, diversity, evenness, and richness) and mite abundance were negatively associated with pH, as detected in other studies. High pH can disrupt soil structure, reducing soil mesofauna abundance and diversity (Yu *et al.,* 2021; Rzeszowski *et al.,* 2017). Collembolan community parameters and the abundance of mites were negatively associated with exposed soil, aligning with their preference for litter layers on the soil surface (Petersen & Luxton, 1982). For example, in vineyard soils, the absence of weed cover appears to have prevented the development of a favourable microclimate for collembolans and mites (Renaud *et al.,* 2004). In contrast, the abundance of collembolans and mites was positively linked to moisture, as also reported in previous studies (Testerink, 1982; Sylvain *et al.,* 2014; Manu *et al.,* 2022). Organic matter and the proportion of legumes in vegetation positively correlated with collembolan community parameters and mite abundance. This is likely because, as with nematodes, increased organic matter promotes microbial growth, which subsequently supports higher populations of microbial consumers such as collembolans and mites (Neilson *et al.,* 2020). The positive relation between legumes and collembolans was also reported in other studies, suggesting that this relation is caused by increased root and microbial biomass, and elevated quantity and quality of plant residues serving as food resources for collembolans (Salamon *et al.,* 2004; Sabais *et al.,* 2011), which was also evident for mite communities (Wissuwa *et al.,* 2012). This study also showed that larger site areas support greater

diversity, evenness, and richness of collembolan communities. A study by Yu *et al.* (2021) highlighted a positive relationship between site area and mesofauna communities. Apart from this study, research directly linking site area with soil community organisms is scarce, especially compared to the wealth of studies on aboveground fauna (Evans *et al.,* 2009; Parris, 2006; Magle, 2009).

In contrast to the other faunal groups, all earthworm community parameters showed a positive relation with pH, consistent with the literature (Tóth *et al.,* 2020; Xie *et al.,* 2018; Baker & Whitby, 2003). Generally, earthworms are scarce or absent in acidic soils (Curry, 1998), likely due to nutrient limitations (e.g., calcium) associated with low pH, which are essential for earthworm survival (Hauxwell *et al.,* 1992). Both abundance and biomass of earthworms were unexpectedly negatively correlated with moisture, a finding contrary to previous studies, that reported a positive relationship between earthworm communities and soil moisture; low moisture levels have been shown to reduce the earthworm weight and even decrease their survival rate (Xie *et al.,* 2018; Berry & Jordan, 2001; Richardson *et al.,* 2009). I hypothesize that the urban greenspaces of Braga consistently maintain adequate soil moisture levels, as the climate in this city is characterized by frequent rainfall, particularly in the autumn when samples were collected, preventing the soil from drying out. As a result, the negative correlation between earthworms and moisture could be due to oversaturation of the soil. Obtained results do not necessarily reflect exceeding soil moisture in these sites at the moment of sampling (see Annex 7), and it is unclear whether effects of previous rainfall or flooding would have carried over to the sampled communities. Indeed, several studies reported that earthworm abundance and biomass is usually reduced by flooding (Ausden *et al.,* 2001; Plum & Filser, 2005). Exposed soils tend to have faster water drainage and retain less moisture (Wang *et al.,* 2013), and this would support earthworm community parameters being positively associated with exposed soils. Further, all earthworm community parameters were negatively correlated with soil organic matter content. If in natural and agricultural ecosystems, earthworm communities typically show a positive relationship with organic matter (Ernst & Emmerling, 2009; Burtelow *et al.,* 1998), a negative relationship has been previously reported in urban areas (Xie *et al.,* 2018). Interestingly, all earthworm community parameters showed a negative correlation with legume cover, which is unusual, as legumes typically support earthworm populations with their nitrogen-rich residues that meet the high nitrogen needs of earthworms (Piotrowska *et al.,* 2013; Eisenhauer *et al.,* 2009). Another unexpected result was that earthworm diversity, evenness and richness tended to be smaller in larger areas, which contradicts previous findings. Earthworm species richness has been associated with larger areas

of green spaces, as these contain more habitats, and thus more potential niches (Xie *et al.,* 2022). However, in this study, only the area was evaluated and not the niche availability and heterogeneity in green spaces.

4.3.3. Influence of the environmental variables on faunal taxonomical diversity

The analyses considering nematode families and genera and earthworm species were done to gain better insights into the nematode and earthworm community structure and how it responds to environmental change. However, a clear pattern related to functional groups for either nematodes or earthworms was not apparent. But, interestingly, in the case of nematodes, environmental factors influenced the omnivores and predators and the plant parasites *Meloidogyne* and *Pratylenchus* in opposite directions.

Meloidogyne and *Pratylenchus* were both positively influenced by pH and the percentage of exposed soil, and interestingly, omnivores and predators, were negatively influenced by those factors. Predators and omnivores are the most sensitive groups to alkaline conditions (Šalamún *et al.,* 2014). As so, higher pH would possibly have decreased the populations of omnivores and predators, which would in turn have increased the population of plant parasitic nematodes, as these higher trophic groups are responsible for their regulation (Khan & Kim, 2007; Kanwar *et al.,* 2021). In fact, higher soil pH has been widely reported to favour the populations of plant-parasitic nematodes, including *Meloidogyne* and *Pratylenchus* (Loewenberg *et al.,* 1960; Melakeberhan *et al.,* 2004; Noronha *et al.,* 2020). The associated exposed soil with these plant parasitic nematodes, may be due to a large burden of plant parasitism imposed by herbivore nematodes that may limit plant establishment and performance, as explained previously (Jones *et al.,* 2013)

This study also revealed that forests exhibited a surprising distinct earthworm composition. Interestingly, all earthworm species were correlated with the same environmental variables, except for a few species, as *Octolasion tyrtaeum* and *Dendrobaena octaedra,* that were correlated with the opposite variables, area site and organic matter. *Dendrobaena octaedra* is commonly found in forests with rich soils, inhabiting in the litter (Terhivuo & Valovirta, 1978; Makarova & Kolesnikova, 2019). However, *Octolasion tyrtaeum* is reported to inhabit a wide range of habitats (Jaenike *et al.,* 1980). Very interestingly, forests only presented these two species, and these species were only present in forests. The multivariate ordination analyses constrained by soil parameters as environmental variables revealed that forests had a distinct earthworm composition and unique environmental conditions. In contrast, the other land uses displayed greater similarities in their earthworm composition and environmental variables. Another interesting finding from this study was that the invasive earthworm *Amynthas corticis* exhibited the same preferences for environmental

variables as other native species. This is particularly concerning because it indicates their potential to invade similar environments.

4.4. Contrasts and Interconnections in Soil Biota Indicators

The interrelationships between nematode, earthworm, mite and collembolan communities revealed intricate dynamics that can significantly influence soil health and ecosystem functioning. These interconnections highlight the delicate balance within soil ecosystems, where changes in the abundance or diversity of one group can significantly impact the others (Bardgett & Van Der Putten, 2014). Understanding these correlations is crucial for developing land management strategies that promote biodiversity and soil health (Bach *et al.,* 2020). The positive correlation between nematode biomass and earthworm richness suggests a potential beneficial interaction between these groups, or responsiveness to similar conditions. In contrast, the negative correlation between collembolan diversity and earthworm diversity may reflect different ecological preferences or indirect competition. Additionally, functional indices like the maturity index, which positively correlated with collembolan abundance, and the structure index, which negatively correlated with earthworm abundance, demonstrate that parameters across faunal groups do not necessarily align with expected patterns of trophic food web structure or maturity.

Despite observing some clear trends, the results from the nematode indices and metabolic footprints exhibited opposing patterns, more specifically the structure index and channel index. The structure index was higher in forests and flower beds, but this did not align with the structure footprints, which showed no significant differences across land uses. Although not statistically significant, forests and unused lands ranked highest in structure footprints, while flower beds had the lowest, as expected. The channel index, a marker of fungal dominance, was also higher in forests, unused lands, and unexpectedly, roadsides. However, the analysis of fungivore and bacterivore footprints revealed that while forests and unused lands had a balanced fungal-to-bacterial ratio, roadsides showed a much higher bacterivore footprint than fungivore footprint. These opposing results between indices and footprints arise from the different methods used to calculate each metric. Diversity and functional indices are based on the relative weighted abundance of different functional guilds of nematodes and are therefore based on nematode numbers (Ferris *et al.*, 2001), whereas footprints provide additional descriptive information on the biomass, metabolic activity, and magnitudes of carbon (C) and energy flow in soil food webs (Ferris, 2010). The nematode ecological indices are useful descriptive tools for assessment of food web and ecosystem condition. Their straightforward calculation and interpretation has

supported their wide use (Du Preez *et al.,* 2022), and especially faunal profiles have been providing rationale for action in soil management (Ferris *et al.,* 2001). However, they cannot provide information on the magnitude or nature of ecosystem functions, a major applicable feature of metabolic footprints (Sanchez-Moreno & Ferris, 2008). Future research should prioritize the use of nematode metabolic footprints over traditional nematode indices, as the indices employed in this study produced inconsistent and puzzling results.

The soil community provides many potential indicators for environmental monitoring in response to various stresses or disturbances. Biological indicators are organisms whose response, in terms of presence/absence, abundance, activity, morphology, physiology or behaviour, gives information on the condition of a habitat or ecosystem (Gerhardt *et al.,* 2002). The organisms examined in this study — nematodes, collembolans, mites and earthworms — are widely recognized as bioindicators, and have been frequently used in ecological research (Ponge *et al.,* 2004; Du Preez *et al.,* 2018; Fründ *et al.,* 2010; Manu *et al.,* 2019). In this study, results on the communities of nematodes, collembolans and mites appear to converge, implying a preference for the same environmental variables – organic matter content, percentage of legumes in the vegetation, moisture and site area - and a preference for the least disturbed land use - forests. However, results on earthworms are distinct, as earthworms seemed to prefer the more disturbed land uses according to results on nematodes, mites and collembolas - lawns and roadsides - and opposing environmental variables – exposed soil and higher pH. In addition to biological indicators, non-biological indicators also play a significant role in assessing soil health. This study highlighted environmental variables with the greatest impact on biodiversity, which could be used to monitoring soil health, including organic matter content, percentage of legumes in vegetation, soil moisture, site area, exposed soil, and pH levels.

The European Commission presented a new EU soil strategy for 2030, with the aim of having all EU soil ecosystems in a healthy condition by 2050 (COM/2021/699) (European Commission, 2021). To achieve this objective, it tabled a proposal for a soil monitoring and resilience directive, laying down measures for monitoring and assessing soil health (COM/2023/416) (European Commission, 2023b). However, concerns have been raised about the indicators chosen to describe and assess soil health. Member States would have an obligation to monitor soil health in each soil district utilizing a proposed set of soil descriptors for this purpose. The pH, organic matter, and moisture levels are already included among the soil health descriptors. Based on the findings of my study, additional observations would enhance the assessment framework

particularly in urban settings: the extent of exposed soil, the percentage of legumes in the vegetation, and the site area. The proposal also suggests that Member States be required to include at least three additional biological indicators in their soil health assessments. My own results on the mismatch of soil quality assessments according to the faunal group analysed supports such requirement.

4.5. Soil functions and Ecosystem services

The importance of soil biodiversity for supporting ecosystem services has been previously reported in natural ecosystems (Delgado-Baquerizo *et al.,* 2020; Delgado-Baquerizo *et al.,* 2016; Wagg *et al.,* 2014; Brockerhoff *et al.,* 2017), however, such reports are scarce for urban greenspaces (Fan *et al*., 2023; Schittko *et al.,* 2022). Inferring about which land use would provide the better ecosystem services based on the present soil communities, can be a complex task, as the different communities of organisms in this study thrived in different land uses. The relationships between biodiversity and ecosystem functions and between biodiversity and ecosystem services are complex (Duncan *et al.,* 2015).

Using metabolic footprints as indicators of the magnitude of the ecosystem services has been suggested by Sánchez-Moreno & Ferris (2018). The mineralization service was higher in flower beds, lawns and roadsides, according to the enrichment, bacterivore and fungal footprint. The disservice of herbivory was higher in flower beds, according to the herbivore footprint. This is considered a disservice as it provides estimates of how much carbon is being lost to pests and diseases (Sánchez-Moreno & Ferris, 2018). The root damage caused by nematodes, combined with suppressed plant defences, often creates entry points for secondary infections by other pests and pathogens. As a result, plant-parasitic nematodes are frequently linked to disease complexes in natural ecosystems (Costa, 2024). Pest and disease pressure can be mitigated by the regulation service, associated with the structure, predator and omnivore footprint. This service was higher in forests and in unused lands and reflects the regulation effects of higher trophic groups in the soil food web (Ferris *et al*., 2012).

According to the nematode, collembolan and mite community analyses, forests hold conditions for enhanced plant growth, primary productivity, nutrient cycling, carbon sequestration, improved decomposition, nitrogen mineralization, soil formation as well as improved pest and disease control and regulation of bacterial and fungal populations (Ferris, 2010; Yeates *et al.,* 2009; Ferris *et al.,* 1998; Filser, 2002; Seastedt & Crossley, 1980; Coleman, 2008). Lawns and roadsides, by contrast, are likely to be poorer in these functions, due to the poorer nematode, collembola and mite communities. Considering the environment variables, soils

with high levels of organic matter, legume percentage in vegetation, moisture, and site area would also promote these functions, while soils with higher pH and exposed soil will be poorer in these functions.

In accordance with the analysis of earthworm community analyses, lawns and roadsides would benefit more from the activity of these "ecosystem engineers," as earthworms mix soil layers, distribute nutrients, and create habitats for other soil organisms through bioturbation (Blouin *et al.,* 2013). In turn, forests would experience less of this earthworm-driven soil transformation and habitat creation, due to the scarcity of these organisms. Assessing the environment variables, soils with high levels of pH and exposed soil would promote these functions, however at the same time these soils also were responsible for promoting the nematode genera *Meloidogyne* and *Pratylenchus,* frequently linked to disease complexes in natural ecosystems (Costa, 2024) and agroecosystems (Jones *et al.,* 2013) while simultaneously negatively impacting higher trophic level nematodes such as omnivores and predators. This will in turn have negative impacts in the providing of ecosystem services.

Based on these results, forests appear to be the most effective land use for promoting a greater range of ecosystem services. Other comparative studies also showed that forests are the main provider of ecosystem services for urban residents, meaning that forest land, although not associated with the basic urban fabric, is essential for cities (Pukowiec-Kurda, 2022). However, this does not diminish the value of ecosystem services provided by other land uses. Rather, it underscores that forests generally offer these services at a greater magnitude. Other green spaces, as shown in this study, support diverse soil organisms and contribute to various ecosystem functions. With improvements, these land uses could further enhance the magnitude of the services they provide. Also, according to this study, exposed soil and high pH levels appear to be the key limiting factors. In this thesis, ecosystem services such as provision and cultural services have not been assessed.

4.6. Urban Management Strategies for Optimizing Ecosystem Services

Urban management strategies have an important impact on soil and its functional role in maintaining ecosystem processes. Improvements in soil management to avoid a reduction in soil fauna diversity and to guarantee suitable soil conditions for these organisms to develop are the only way to ensure long-term soil functioning and the provider of ecosystem services (Ertiban, 2019). Although forests were key providers of a range of ecosystem services, they are often cut down to make way for housing and infrastruture construction (Pukowiec-Kurda & Vavrouchowa, 2020). As so, maintaining them and, as far as possible, increasing their

area should be a priority for city managers, especially in spaces with the largest buildings and the highest population density (Giannico *et al.,* 2021).

The limiting factors, exposed soil and high pH levels, highlight the importance to carefully manage the residues of constructions during urban development to prevent long-term soil degradation and ensure healthy soil communities, as the high pH in urban soils is frequently connected to artificial product wastes that are buried into soils during city constructions (Liu *et al.,* 2021). The management of urban areas should also prioritize strategies to minimize exposed soils in green spaces by establishing and maintaining vegetative soil cover. Also, practices should be implemented to enhance organic matter content in specific green spaces when physicochemical analyses indicates that such improvements are needed. However, caution should be taken when choosing the type of fertilizer to use. Nutrients in mineral fertilizers cannot be used by the brown energy channel of the soil food web, and thus promote the green energy channel, favouring plant-parasitic nematodes (Costa, 2024). As a viable substitute, organic fertilization should be included in these management practices. Organic fertilization can increase soil organic matter content, improving soil structure and promoting soil food web biomass and complexity, while reducing plant parasitic nematode populations (Schrama *et al.,* 2018, Costa, 2024). Also, efficient irrigation practices should be incorporated to ensure optimized water levels in organic soils so that the structure and composition of such soils are not negatively affected. All these measures are already encompassed in the soil monitoring and resilience directive (COM/2023/416) (European Commission, 2023b). However, based on the findings of my study, additional management strategies should be considered for inclusion: integration of legumes in the vegetation of greenspaces and the expansion of green spaces. This could include the creation of ecological corridors to improve the entire urban area (Peng *et al.,* 2017) or efforts to preserve and avoid further reduction of existing green areas. Additionally, and particularly concerning, flowerbeds in this study showed an exceptional proportion of plant-parasitic nematodes. Therefore, effective measures should be implemented to control these nematodes in these greenspaces. Synthetic pesticides can broadly affect the soil food web due to their high environmental toxicity, and have non-target, leading to unintended and often unforeseen consequences, even after thorough testing before release. Nematicides can harm not only plant-parasitic nematodes but also beneficial organisms, such as free-living nematodes (Timper *et al.,* 2012). Promising new nematicides are being developed with a focus on assessing their impact on non-target soil organisms to minimize these adverse effects, but nematode management should ideally resort to alternative, environmentally-friendly methods supported by agroecology research (Costa, 2024). Also, in this study, the invasive earthworm

Amynthas corticis were found in flower beds. Earthworm cocoons can be dispersed between flower beds attached to gardening tools, equipment, soles of boots and shoes (Dupont *et al.*, 2012, Baumann *et al.,* 2024). As so, special cautions should be implemented, such as purchasing planting material and mulch from certified sources to avoid contamination, and cleaning soil and other debris from equipment, boots, and gardening tools before moving to new sites to prevent the spread of this invasive species (Baumann *et al.,* 2024).

4.7. Conclusions and future perspectives

The results of this study together showed that managed sites, such as lawns and roadsides, were nutrient-rich but disturbed, favouring bacterial over fungal decomposition, while flower beds were dominated by plant-parasitic nematodes. Non-managed sites, like forests and unused lands, generally displayed healthier ecosystems. However, even unused lands were dominated by plant-parasitic nematodes. Forests had the most balanced and stable ecological state, supporting healthier communities of nematodes, collembolans, and mites, while earthworms thrived in lawns and roadsides. The results also revealed that collembolans, mites, and nematodes shared preferences for similar environmental variables, including organic matter, percentage of legumes in vegetation, moisture, and site area. Interestingly, earthworms exhibited opposite preferences, favouring soils soil with high pH and exposed soil. Also, the results indicated that forests were the most effective land use type in promoting a broader range of ecosystem services.

For future research, several additional steps could be taken to improve the results. One potential limitation of this study was that the samples were collected exclusively in autumn, a season characterized by greater accumulation of leaf litter and increased rainfall. Several research in greenspaces has demonstrated that seasonal variability significantly influences soil community composition (Hu *et al.,* 2024; Huang *et al.,* 2020). To address this, future studies should include samples from at least two different seasons, such as autumn and spring, to account for seasonal variability.

A finer taxonomical classification of mite communities would have provided a stronger dataset for analysis, and this study only evaluated their abundance. Numerous studies have demonstrated that mites are highly sensitive to disturbances, making a comprehensive examination of their diversity essential (Behan-Pelletier, 1999; Manu *et al.,* 2019; Ivan & Vasiliu, 2009). Also, although the most widely used technique for quantitative sampling of earthworms is hand digging and hand sorting (Satchel 1969; Edwards & Lofty 1977; Lee 1985), this technique has some limitations and may not be representing the whole community. Various

studies reported that the combination of chemical expellant plus hand-sorting is preferred (Gutiérrez-López *et al.,* 2016; Rombke *et al.,* 2016). Future research should implement this technique, as it could potentially reveal any contradictory results in the earthworm data found in this study.

Conflicting results and limited interpretations could have benefitted from the collection and analysis of more environmental variables from the sites. Vegetation is a major player in soil communities, and ultimately the source of most of the food entering the soil food web. This study only analysed the functional composition of herbaceous vegetation. Therefore, future research should consider other factors, such as species-level identification of vegetation in different strata, and include quantification of root fragments and biomass, and an analysis and quantification of leaf litter. Decomposition could have been promptly determined by the well-established tea bag method (Keuskamp *et al.,* 2013), and microorganism communities analysed in more detail using, e.g. metagenomic approaches. Moreover, this study did not investigate soil contamination by heavy metals, which is a widespread problem in urban settings, and could have directly influenced the soil biota. Finally, it is essential that future studies examine landscape factors such as habitat fragment area, distance to natural areas, and habitat connectivity. While these factors have been extensively studied for aboveground communities, they have received little attention in belowground studies. However, recent investigations have indicated that these landscape variables also influence earthworm communities (Xie *et al.,* 2018), microorganisms (Liu *et al.,* 2022), and both mites and collembolans (Piano *et al.,* 2020).

This study allowed to advance the ecological theory in the soil component in urban environments, contributing to a better knowledge frame to inform on city management and planning decisions. This is well aligned with the EU Mission "A Soil Deal for Europe" (European Commission, 2023a), one of five Missions funded under the EU Research and Innovation Programme Horizon Europe. Mission Soil has eight specific objectives, which are complementary with the results of my study: reduce land degradation relating to desertification; conserve and increase soil organic carbon stocks; no net soil sealing and increase reuse of urban soils; reduce soil pollution and enhance restoration; prevent erosion; improve soil structure to enhance soil habitat quality for soil biota and crops; reduce the EU global footprint on soils and increase soil literacy in society across Member States. Thus, it is imperative that this line of research is strengthened, expanded and replicated in other cities, to better establish the links between soil biodiversity and function in urban ecosystems, and to be able to infer on the magnitude of anthropogenic pressures and their mitigating factors.

This will ultimately allow the design of appropriate measures and the implementation of scientifically-sound policies for urban green spaces, that increase their resilience to impacts and promote their provision of crucial ecosystem services.

5. References

Addison, J. A. (2009). Distribution and impacts of invasive earthworms in Canadian forest ecosystems. *Biological Invasions*, *11*, 59-79.

Afzal, S., Nesar, H., Imran, Z., & Ahmad, W. (2023). Change in land-use from natural forest impacts functional composition and metabolic footprint of soil nematode community in Western Himalayas. *Acta Ecologica Sinica*, *43*(5), 842-852.

Ahmed, N., & Al-Mutairi, K. A. (2022). Earthworms effect on microbial population and soil fertility as well as their interaction with agriculture practices. *Sustainability*, *14*(13), 7803.

Aislabie, J., Deslippe, J. R., & Dymond, J. (2013). Soil microbes and their contribution to soil services. *Ecosystem services in New Zealand–conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand*, *1*(12), 143-161.

Allison, S. D. (2006). Brown ground: a soil carbon analogue for the green world hypothesis?. *The American Naturalist*, *167*(5), 619-627.

Amossé, J., Dózsa-Farkas, K., Boros, G., Rochat, G., Sandoz, G., Fournier, B., Mitchell, E. A. D., & Le Bayon, R. C. (2016). Patterns of earthworm, enchytraeid and nematode diversity and community structure in urban soils of different ages. *European Journal of Soil Biology*, *73*, 46-58.

Anderson, J. M., Ineson, P., & Huish, S. A. (1983). Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodlands. *Soil Biology and Biochemistry*, *15*(4), 463-467.

Asabere, S. B., Zeppenfeld, T., Nketia, K. A., & Sauer, D. (2018). Urbanization leads to increases in pH, carbonate, and soil organic matter stocks of arable soils of Kumasi, Ghana (West Africa). *Frontiers in Environmental Science*, *6*, 119.

Ausden, M., Sutherland, W. J., & James, R. (2001). The effects of flooding lowland wet grassland on soil macroinvertebrate prey of breeding wading birds. *Journal of Applied ecology*, *38*(2), 320-338.

Bach, E. M., Ramirez, K. S., Fraser, T. D., & Wall, D. H. (2020). Soil biodiversity integrates solutions for a sustainable future. *Sustainability*, *12*(7), 2662.

Bachelier, G., 1986. La vie animale dans le sol. ORSTOM, Paris. pp. 171–196.

Baker, G. H., & Whitby, W. A. (2003). Soil pH preferences and the influences of soil type and temperature on the survival and growth of Aporrectodea longa (Lumbricidae): the 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. *Pedobiologia*, *47*(5-6), 745-753.

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), 505-511.

Baumann, T. T., Frelich, L. E., Van Riper, L. C., & Yoo, K. (2024). Anthropogenic transport mechanisms of invasive European earthworms: a review. *Biological Invasions*, *26*(11), 3563-3586.

Beck, L., Römbke, J., Breure, A. M., & Mulder, C. (2005). Considerations for the use of soil ecological classification and assessment concepts in soil protection. *Ecotoxicology and Environmental Safety*, *62*(2), 189-200.

Behan-Pelletier, V. M. (1999). Oribatid mite biodiversity in agroecosystems: role for bioindication. *Agriculture, ecosystems & environment*, *74*(1-3), 411-423.

Bellitürk, K., Görres, J. H., Kunkle, J., & Melnichuk, R. D. S. (2015). Can commercial mulches be reservoirs of invasive earthworms? Promotion of ligninolytic enzyme activity and survival of Amynthas agrestis (Goto and Hatai, 1899). *Applied Soil Ecology*, *87*, 27-31.

Beninde, J., Veith, M., & Hochkirch, A. (2015). Biodiversity in cities needs space: a meta-analysis of factors determining intra-urban biodiversity variation. *Ecology letters*, *18*(6), 581-592.

Berry, E. C., & Jordan, D. (2001). Temperature and soil moisture content effects on the growth of Lumbricus terrestris (Oligochaeta: Lumbricidae) under laboratory conditions. *Soil Biology and Biochemistry*, *33*(1), 133-136.

Biasioli, M., Barberis, R., & Ajmone-Marsan, F. (2006). The influence of a large city on some soil properties and metals content. *Science of the Total Environment*, *356*(1-3), 154-164.

Birkhofer, K., Schöning, I., Alt, F., Herold, N., Klarner, B., Maraun, M., Marhan, S., Oelmann, Y., Wubet, T., Yurkov, A., Begerow, D., Berner, D., Buscot, F., Daniel, R., Diekötter, T., Ehnes, R. B., Erdmann, G., Fischer, C., Foesel, B., Groh, J., Gutknecht, J., Kandeler, E., Lang, C., Lohaus, G., Meyer, A., Nacke, H., Näther, A., Overmann, J., Polle, A., Pollierer, M. M., Scheu, S., Schloter, M., Schulze, E.D., Schulze, W., Weinert, J., Weisser, W. W., Wolters, V., & Schrumpf, M. (2012). General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types.

Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D., & Brun, J. J. (2013). A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, *64*(2), 161-182.

Boeddinghaus, R. S., Marhan, S., Berner, D., Boch, S., Fischer, M., Hölzel, N., Kattge, J., Klaus, V. H., Kleinebecker, T., Oelmann, Y., Prati, D., Schäfer, D., Schöning, I., Schrumpf, M., Sorkau, E., Kandeler, E., & Manning, P. (2019). Plant functional trait shifts explain concurrent changes in the structure and function of grassland soil microbial communities. *Journal of Ecology*, *107*(5), 2197-2210.

Bokhorst, S., Huiskes, A., Convey, P., Van Bodegom, P. M., & Aerts, R. (2008). Climate change effects on soil arthropod communities from the Falkland Islands and the Maritime Antarctic. *Soil Biology and Biochemistry*, *40*(7), 1547-1556.

Bolger, T., Kenny, J., & Arroyo, J. (2013). The Collembola fauna of Irish forests–a comparison between forest type and microhabitats within the forests. *Soil Organisms*, *85*(1), 61-67.

Bolund, P., & Hunhammar, S. (1999). Ecosystem services in urban areas. *Ecological economics*, *29*(2), 293-301.

Bongers, T. (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia*, *83*, 14-19.

Bongers, T., & Bongers, M. (1998). Functional diversity of nematodes. *Applied soil ecology*, *10*(3), 239-251.

Bongers, T., & Ferris, H. (1999). Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology & Evolution*, *14*(6), 224-228.

Brockerhoff, E. G., Barbaro, L., Castagneyrol, B., Forrester, D. I., Gardiner, B., González-Olabarria, J. R., Lyver, P. O'B., Meurisse, N., Oxbrough, A., Taki, H., Thompson, I. D., van der Plas, F., & Jactel, H. (2017). Forest biodiversity, ecosystem functioning and the provision of ecosystem services. *Biodiversity and Conservation*, *26*, 3005-3035.

Brussaard, L. (1997). Biodiversity and ecosystem functioning in soil. *Ambio*, 563-570.

Burtelow, A. E., Bohlen, P. J., & Groffman, P. M. (1998). Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. *Applied Soil Ecology*, *9*(1-3), 197-202.

Câmara Municipal de Braga. (n.d.). *Portal oficial da Câmara Municipal de Braga*. https://www.cm-braga.pt/pt

Cardoso, M. S., Pedrosa, E. M., Rolim, M. M., Oliveira, L. S., & Santos, A. N. (2016). Relationship between nematode assemblages and physical properties across land use types. *Tropical Plant Pathology*, *41*, 107-114.

Carrier, J. A., & Beebee, T. J. (2003). Recent, substantial, and unexplained declines of the common toad Bufo bufo in lowland England. *Biological Conservation*, *111*(3), 395-399.

Čerevková, A. (2006). Nematode communities in three types of grassland in the Slovak Republic. *Helminthologia*, *43*, 171-176.

Čerevková, A., Renčo, M., Miklisová, D., & Gömöryová, E. (2021). Soil nematode communities in managed and natural temperate forest. *Diversity*, *13*(7), 327.

Cesarz, S., Reich, P. B., Scheu, S., Ruess, L., Schaefer, M., & Eisenhauer, N. (2015). Nematode functional guilds, not trophic groups, reflect shifts in soil food webs and processes in response to interacting global change factors. *Pedobiologia*, *58*(1), 23-32.

Chauhan, R. P. (2014). Role of earthworms in soil fertility and factors affecting their population dynamics: a review. *International Journal of Research*, *1*(6), 642-649.

Chen, Y., Martinez, A., Cleavenger, S., Rudolph, J., & Barberán, A. (2021). Changes in soil microbial communities across an urbanization gradient: A local-scale temporal study in the arid southwestern USA. *Microorganisms*, *9*(7), 1470.

Coleman, D. C. (2008). From peds to paradoxes: linkages between soil biota and their influences on ecological processes. *Soil Biology and Biochemistry*, *40*(2), 271-289.

Costa, S. R. (2024). Ecology. In R. N. Perry, M. Moens, & J. T. Jones (Eds.), Plant nematology (pp. 316–339). CABI.

Costa, S. R., van der Putten, W. H., & Kerry, B. R. (2011). Microbial ecology and nematode control in natural ecosystems. In *Biological control of plant-parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms* (pp. 39-64). Dordrecht: Springer Netherlands.

Crommentuijn, T., Brils, J., & Vanstraalen, N. M. (1993). Influence of cadmium on life-history characteristics of Folsomia candida (Willem) in an artificial soil substrate. *Ecotoxicology and Environmental Safety*, *26*(2), 216-227.

Crooks, J. A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos*, *97*(2), 153-166.

Curry JP (1998) Factors affecting earthworm abundance in soils. In: Edwards CA (ed) Earthworm ecology. St. Lucie Press, Boca Raton, Fla., pp 37–64

da Silva, P. M., Carvalho, F., Dirilgen, T., Stone, D., Creamer, R., Bolger, T., & Sousa, J. P. (2016). Traits of collembolan life-form indicate land use types and soil properties across an European transect. *Applied Soil Ecology*, *97*, 69-77.

de Carvalho, F. C. (2012). Efeito de diferentes tipos de gestão em olivais nos microartrópodes de solo usando uma abordagem funcional (Master's thesis, Universidade de Coimbra (Portugal)).

Dean, W. E. (1974). Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition; comparison with other methods. *Journal of Sedimentary Research*, *44*(1), 242-248.

Decaëns, T. (2010). Macroecological patterns in soil communities. *Global Ecology and Biogeography*, *19*(3), 287-302.

Delgado de la Flor, Y. A., Perry, K. I., Turo, K. J., Parker, D. M., Thompson, J. L., & Gardiner, M. M. (2020). Local and landscape-scale environmental filters drive the functional diversity and taxonomic composition of spiders across urban greenspaces. *Journal of Applied Ecology*, *57*(8), 1570-1580.

Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., Berdurgo, M., Campbell, C. D., & Singh, B. K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature communications*, *7*(1), 10541.

Delgado-Baquerizo, M., Reich, P. B., Trivedi, C., Eldridge, D. J., Abades, S., Alfaro, F. D., Bastida, F., Berhe, A. A., Cutler, N. A., Gallardo, A., García-Velázquez, L., Hart, S. C., Hayes, P. E., He, J.-Z., Hseu, Z.-Y., Hu, H.-W., Kirchmair, M., Neuhauser, S., Pérez, C. A., Reed, S. C., Santos, F., Sullivan, B. W., Trivedi, P., Wang, J.-T., Weber-Grullon, L., Williams, M. A., & Singh, B. K. (2020). Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature ecology & evolution*, *4*(2), 210-220.

Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., Hacquard, S., Hervé, V., Labbé, J., Lastovetsky, O. A., Mieszkin, S., Millet, L. J., Vajna, B., Junier, P., Bonfante, P., Krom, B. P., Olsson, S., van Elsas, J. D., & Wick, L. Y. (2018). Bacterial–fungal interactions: ecology, mechanisms and challenges. *FEMS microbiology reviews*, *42*(3), 335-352.

Dmowska, E., & Kozlowska, J. (1988). Communities of nematodes in soil treated with semi-liquid manure. *Pedobiologia*, *32*(5-6), 323-330.

Dominati, E., Patterson, M., & Mackay, A. (2010). A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecological economics*, *69*(9), 1858-1868.

Du Preez, G. C., Daneel, M. S., Wepener, V., & Fourie, H. (2018). Beneficial nematodes as bioindicators of ecosystem health in irrigated soils. *Applied Soil Ecology*, *132*, 155-168.

Du Preez, G., Daneel, M., De Goede, R., Du Toit, M. J., Ferris, H., Fourie, H., Geisen, S., Kakouli-Duarte, T., Korthals, G., Sánchez-Moreno, S., & Schmidt, J. H. (2022). Nematode-based indices in soil ecology: Application, utility, and future directions. *Soil Biology and Biochemistry*, *169*, 108640.

Duarte, A. C., Guerra, C. A., Cano-Díaz, C., Zeiss, R., Carvalho-Santos, C., Carvalho, R. P., & Costa, S. R. (2024). Effects of protected areas on soil nematode communities in forests of the North of Portugal. *Biodiversity and Conservation*, *33*(1), 73-89.

Duncan, C., Thompson, J. R., & Pettorelli, N. (2015). The quest for a mechanistic understanding of biodiversity–ecosystem services relationships. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1817), 20151348.

Dupont, L., Decaëns, T., Lapied, E., Chassany, V., Marichal, R., Dubs, F., Maillot, M., & Roy, V. (2012). Genetic signature of accidental transfer of the peregrine earthworm Pontoscolex corethrurus (Clitellata, Glossoscolecidae) in French Guiana. *European Journal of Soil Biology*, *53*, 70-75.

Edwards, C. A., & Bohlen, P. J. (1996). *Biology and ecology of earthworms* (Vol. 3). Springer Science & Business Media.

Eisenhauer, N., Milcu, A., Sabais, A. C., Bessler, H., Weigelt, A., Engels, C., & Scheu, S. (2009). Plant community impacts on the structure of earthworm communities depend on season and change with time. *Soil Biology and Biochemistry*, *41*(12), 2430-2443.

Eldridge, D. J., Cui, H., Ding, J., Berdugo, M., Sáez-Sandino, T., Duran, J., Gaitan, J., Blanco-Pastor, J. L., Rodríguez, A., Plaza, C., Alfaro, F., Teixido, A. L., Abades, S., Bamigboye, A. R., Peñaloza-Bojacá, G. F., Grebenc, T., Nahberger, T. U., Illán, J. G., Liu, Y.-R., Makhalanyane, T. P., Rey, A., Siebe, C., Sun, W., Trivedi, P., Verma, J. P., Wang, L., Wang, J., Wang, T., Zaady, E., Zhou, X., Zhou, X.-Q., & Delgado-Baquerizo, M. (2024). Urban greenspaces and nearby natural areas support similar levels of soil ecosystem services. *npj Urban Sustainability*, *4*(1), 15.

Epp Schmidt, D. J., Pouyat, R., Szlavecz, K., Setälä, H., Kotze, D. J., Yesilonis, I., Cilliers, S., Hornung, E., Dombos, M., & Yarwood, S. A. (2017). Urbanization erodes ectomycorrhizal fungal diversity and may cause microbial communities to converge. *Nature ecology & evolution*, *1*(5), 0123.

Ernst, G., & Emmerling, C. (2009). Impact of five different tillage systems on soil organic carbon content and the density, biomass, and community composition of earthworms after a ten year period. *European Journal of Soil Biology*, *45*(3), 247-251.

Ertiban, S. M. (2019). Soil fauna as webmasters, engineers and bioindicators in ecosystems: implications for conservation ecology and sustainable agriculture. *American Journal of Life Sciences*, *7*(1), 17-26.

European Commission. (2021). Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, EU Soil Strategy for 2030 – Reaping the benefits of healthy soils for people, food, nature and climate, COM (2021) 699 final of 17.11.2021.

European Commission. (2023a). *A soil deal for Europe*. Retrieved January 4, 2025, from https://research-and-innovation.ec.europa.eu/funding/funding-opportunities/funding-programmes-and-open-calls/horizon-europe/eu-missions-horizon-europe/soil-deal-europe_en

European Commission. (2023b). European Commission, Proposal for a Directive of the European Parliament and of the Council on Soil Monitoring and Resilience (Soil Monitoring Law) COM/2023/416 final.

Evans, K. L., Newson, S. E., & Gaston, K. J. (2009). Habitat influences on urban avian assemblages. *Ibis*, *151*(1), 19-39.

Fan, K., Chu, H., Eldridge, D. J., Gaitan, J. J., Liu, Y. R., Sokoya, B., Wang, J. T., Hu, H. W., He, J. Z., Sun, W., Cui, H., Alfaro, F. D., Abades, S., Bastida, F., Díaz-López, M., Bamigboye, A. R., Berdugo, M., Blanco-Pastor, J. L., Grebenc, T., Duran, J., Illán, J. G., Makhalanyane, T. P., Mukherjee, A., Nahberger, T. U., Peñaloza-Bojacá, G. F., Plaza, C., Verma, J. P., Rey, A., Rodríguez, A., Siebe, C., Teixido, A. L., Trivedi, P., Wang, L., Wang, J., Yang, T., Zhou, X. Q., Zhou, X., Zaady, E., Tedersoo, L., & Delgado-Baquerizo, M. (2023). Soil biodiversity supports the delivery of multiple ecosystem functions in urban greenspaces. *Nature Ecology & Evolution*, *7*(1), 113-126.

Fenoglio, M. S., Rossetti, M. R., & Videla, M. (2020). Negative effects of urbanization on terrestrial arthropod communities: A meta-analysis. *Global Ecology and Biogeography*, *29*(8), 1412-1429.

Ferris, H. (2010). Contribution of nematodes to the structure and function of the soil food web. *Journal of nematology*, *42*(1), 63.

Ferris, H. (2010). Form and function: metabolic footprints of nematodes in the soil food web. *European Journal of Soil Biology*, *46*(2), 97-104.

Ferris, H. (2011). Key to families (and Subfamilies) of plant and soil nematodes. *California: University of California, Davis. URL: http://nemaplex. ucdavis. edu/Taxadata/Famkey. htm*.

Ferris, H. Nematode Ecophysiological Parameters. http://nemaplex.ucdavis. edu/Ecology/EcophysiologyParms/EcoParameterMenu.html (2018)

Ferris, H. V. R. C., Venette, R. C., Van Der Meulen, H. R., & Lau, S. S. (1998). Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement. *Plant and Soil*, *203*, 159-171.

Ferris, H., Bongers, T., & de Goede, R. G. (2001). A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied soil ecology*, *18*(1), 13-29.

Ferris, H., Sánchez-Moreno, S., & Brennan, E. B. (2012). Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. *Applied Soil Ecology*, *61*, 16-25.

Filser, J. (2002). The role of Collembola in carbon and nitrogen cycling in soil: Proceedings of the Xth international Colloquium on Apterygota, České Budějovice 2000: Apterygota at the Beginning of the Third Millennium. *Pedobiologia*, *46*(3-4), 234-245.

Fox, J., and Weisberg, S. (2019). An Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Francini, G., Hui, N., Jumpponen, A., Kotze, D. J., Romantschuk, M., Allen, J. A., & Setälä, H. (2018). Soil biota in boreal urban greenspace: Responses to plant type and age. *Soil Biology and Biochemistry*, *118*, 145-155.

Fründ, H. C., Graefe, U., & Tischer, S. (2010). Earthworms as bioindicators of soil quality. In *Biology of earthworms* (pp. 261-278). Berlin, Heidelberg: Springer Berlin Heidelberg.

Fuller, R. A., Irvine, K. N., Devine-Wright, P., Warren, P. H., & Gaston, K. J. (2007). Psychological benefits of greenspace increase with biodiversity. *Biology letters*, *3*(4), 390-394.

Gan, H., & Wickings, K. (2020). Root herbivory and soil carbon cycling: shedding "green" light onto a "brown" world. *Soil Biology and Biochemistry*, *150*, 107972.

Georgieva, S. S., McGrath, S. P., Hooper, D. J., & Chambers, B. S. (2002). Nematode communities under stress: the long-term effects of heavy metals in soil treated with sewage sludge. *Applied soil ecology*, *20*(1), 27-42.

Gerhardt, A. (2002). Bioindicator species and their use in biomonitoring. *Environmental monitoring*, *1*, 77-123.

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., & Hättenschwiler, S. (2010). Diversity meets decomposition. *Trends in ecology & evolution*, *25*(6), 372-380.

Giannico, V., Spano, G., Elia, M., D'Este, M., Sanesi, G., & Lafortezza, R. (2021). Green spaces, quality of life, and citizen perception in European cities. *Environmental research*, *196*, 110922.

Goddard, M. A., Dougill, A. J., & Benton, T. G. (2010). Scaling up from gardens: biodiversity conservation in urban environments. *Trends in ecology & evolution*, *25*(2), 90-98.

Gong, X., Qiao, Z., Yao, H., Zhao, D., Eisenhauer, N., Scheu, S., Liang, C., Liu, M., Zhu, Y.G., & Sun, X. (2024). Urbanization simplifies soil nematode communities and coincides with decreased ecosystem stability. *Soil Biology and Biochemistry*, *190*, 109297.

Gong, X., Sun, X., Thakur, M. P., Qiao, Z., Yao, H., Liu, M., Scheu, S., & Zhu, Y. G. (2023). Climate and edaphic factors drive soil nematode diversity and community composition in urban ecosystems. *Soil Biology and Biochemistry*, *180*, 109010.

Goodall, D. W. (1952). Some considerations in the use of point quadrats for the analysis of vegetation. *Australian Journal of Biological Sciences*, *5*(1), 1-41.

Görres, J. H., & Melnichuk, R. D. (2012). Asian invasive earthworms of the genus Amynthas Kinberg in Vermont. *Northeastern Naturalist*, *19*(2), 313-322.

Green, O. O., Garmestani, A. S., Albro, S., Ban, N. C., Berland, A., Burkman, C. E., Gardiner, M. M., Gunderson, L., Hopton, M. E., Schoon, M. L., & Shuster, W. D. (2016). Adaptive governance to promote ecosystem services in urban green spaces. *Urban ecosystems*, *19*, 77-93.

Greiner, H. G., Kashian, D. R., & Tiegs, S. D. (2012). Impacts of invasive Asian (Amynthas hilgendorfi) and European (Lumbricus rubellus) earthworms in a North American temperate deciduous forest. *Biological Invasions*, *14*, 2017-2027.

Guilland, C., Maron, P. A., Damas, O., & Ranjard, L. (2018). Biodiversity of urban soils for sustainable cities. *Environmental chemistry letters*, *16*, 1267-1282.

Güneralp, B., & Seto, K. C. (2013). Futures of global urban expansion: uncertainties and implications for biodiversity conservation. *Environmental Research Letters*, *8*(1), 014025.

Gurr, G. M., Wratten, S. D., & Luna, J. M. (2003). Multi-function agricultural biodiversity: pest management and other benefits. *Basic and Applied Ecology*, *4*(2), 107-116.

Gutiérrez-López, M., Moreno, G., Trigo, D., Juárez, E., Jesús, J. B., & Cosín, D. J. D. (2016). The efficiency of earthworm extraction methods is determined by species and soil properties in the Mediterranean communities of Central-Western Spain. *European Journal of Soil Biology*, *73*, 59-68.

Háněl, L. (2001). Succession of soil nematodes in pine forests on coal-mining sands near Cottbus, Germany. *Applied Soil Ecology*, *16*(1), 23-34.

Hauxwell, J., Krapfl, H., Lamb, J., & Maurer, B. (1992). Earthworm preference: analzying the effects of soil moisture, pH, and calcium levels on the distribution of Lumbricus rubellus.

Hazelhoff, L., Van Hoof, P., Imeson, A. C., & Kwaad, F. J. P. M. (1981). The exposure of forest soil to erosion by earthworms. *Earth Surface Processes and Landforms*, *6*(3-4), 235-250.

Herbert, I. N., Svendsen, C., Hankard, P. K., & Spurgeon, D. J. (2004). Comparison of instantaneous rate of population increase and critical-effect estimates in Folsomia candida exposed to four toxicants. *Ecotoxicology and Environmental Safety*, *57*(2), 175-183.

Herrmann, D. L., Schwarz, K., Shuster, W. D., Berland, A., Chaffin, B. C., Garmestani, A. S., & Hopton, M. E. (2016). Ecology for the shrinking city. *BioScience*, *66*(11), 965-973.

Hishi, T., Fujimaki, R., McGonigle, T. P., & Takeda, H. (2008). Relationships among fine roots, fungal hyphae and soil microarthropods among different soil microhabitats in a temperate coniferous forest of Chamaecyparis obtusa. *European Journal of soil biology*, *44*(5-6), 473-477.

Hopkin, S. P. (1997). *Biology of the springtails:(Insecta: Collembola)*. OUP Oxford.

Howard, P. J. A., & Howard, D. M. (1990). Use of organic carbon and loss-on-ignition to estimate soil organic matter in different soil types and horizons. *Biology and Fertility of soils*, *9*, 306-310.

Hu, J., Chen, G., Wang, F., Hassan, W. M., Cai, M., Fan, W., & Yuan, X. (2024). Nematodes as biological indicators of urban heat island effects in the Chongqing area urban green spaces. *Ecological Indicators*, *158*, 111439.

Huang, Y., Yesilonis, I., & Szlavecz, K. (2020). Soil microarthropod communities of urban green spaces in Baltimore, Maryland, USA. *Urban Forestry & Urban Greening*, *53*, 126676.

Ingham, R. E., Trofymow, J. A., Ingham, E. R., & Coleman, D. C. (1985). Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological monographs*, *55*(1), 119-140.

Ivan, O., & Vasiliu, N. A. (2009). Oribatid mites (Acari, Oribatida)-bioindicators of forest soils pollution with heavy metals and fluorine. *Annals of Forest Research*, *52*, 11-18.

Jaenike, J., Parker Jr, E. D., & Selander, R. K. (1980). Clonal niche structure in the parthenogenetic earthworm Octolasion tyrtaeum. *The American Naturalist*, *116*(2), 196-205.

Jeffery S, Gardi C, Jones A, Montanarella L, Marmo L, Miko L, Ritz K, Peres G, Römbke J, van der Putten W (2010) European atlas of soil biodiversity. European Commission, Brussels

Jim, C. Y. (1998). Physical and chemical properties of a Hong Kong roadside soil in relation to urban tree growth. *Urban Ecosystems*, *2*, 171-181.

Joimel, S., Schwartz, C., Hedde, M., Kiyota, S., Krogh, P. H., Nahmani, J., Pérès, G., Vergnes, A., & Cortet, J. (2017). Urban and industrial land uses have a higher soil biological quality than expected from physicochemical quality. *Science of the Total Environment*, *584*, 614-621.

Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J. E., Wesemael, W. M. L., & Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular plant pathology*, *14*(9), 946-961.

Kanianska, R., Jaďuďová, J., Makovníková, J., & Kizeková, M. (2016). Assessment of relationships between earthworms and soil abiotic and biotic factors as a tool in sustainable agricultural. *Sustainability*, *8*(9), 906.

Kanwar, R. S., Patil, J. A., & Yadav, S. (2021). Prospects of using predatory nematodes in biological control for plant parasitic nematodes–a review. *Biological Control*, *160*, 104668.

Keith, A. M., Brooker, R. W., Osler, G. H., Chapman, S. J., Burslem, D. F., & Van Der Wal, R. (2009). Strong impacts of belowground tree inputs on soil nematode trophic composition. *Soil Biology and Biochemistry*, *41*(6), 1060-1065.

Keuskamp, J. A., Dingemans, B. J., Lehtinen, T., Sarneel, J. M., & Hefting, M. M. (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution*, *4*(11), 1070-1075.

Khan, Z., & Kim, Y. H. (2007). A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. *Applied soil ecology*, *35*(2), 370-379.

Kibblewhite, M. G., Ritz, K., & Swift, M. J. (2008). Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1492), 685-701.

Kitagami, Y., Kanzaki, N., & Matsuda, Y. (2017). Distribution and community structure of soil nematodes in coastal Japanese pine forests were shaped by harsh environmental conditions. *Applied Soil Ecology*, *119*, 91-98.

Kladivko, E. (2003). Influence of agricultural practices on earthworm populations. In *Proceedings of the Crop Protection Technology Conference, University of Illinois, Chicago*.

Lattaud, C., Mora, P., Garvín, M. H., Locati, S., & Rouland, C. (1999). Enzymatic digestive capabilities in geophagous earthworms-origin and activities of cellulolytic enzymes. *Pedobiologia*, *43*(6), 842-850.

Lavelle, P. (1983). The structure of earthworm communities. In *Earthworm ecology: from Darwin to vermiculture* (pp. 449-466). Dordrecht: Springer Netherlands.

Lavelle, P., & Spain, A. V. (2001). Soil organisms. *Soil ecology*, 201-356.

Lazarova, S., Coyne, D., Rodriguez, M. G., Peteira, B., & Ciancio, A. (2021). Functional diversity of soil nematodes in relation to the impact of agriculture—a review. *Diversity*, *13*(2), 64.

Lee, K. E. (1985). Earthworms; their ecology and relationships with soils and land use.

Lee, K. E., & Foster, R. C. (1991). Soil fauna and soil structure. *Soil Research*, *29*(6), 745-775.

Li, L., Tian, J., Luo, Z., Li, L., Niu, Y., Haider, F. U., Nian, L., Zhang, Y., He, R., & Liu, J. (2024). Effects of Perennial Alfalfa on the Structure and Function of Soil Micro-Food Webs in the Loess Plateau. *Microorganisms*, *12*(11), 2268.

Li, X., Liu, T., Li, H., Geisen, S., Hu, F., & Liu, M. (2022). Management effects on soil nematode abundance differ among functional groups and land-use types at a global scale. *Journal of Animal Ecology*, *91*(9), 1770-1780.

Lišková, M., Čerevková, A., & Háněl, L. (2008). Nematode communities of forest ecosystems in. *Russian Journal of Nematology*, *16*(2), 129-142.

Liu, L., Barberán, A., Gao, C., Zhang, Z., Wang, M., Wurzburger, N., Wang, X., Zhang, R., Li, J., & Zhang, J. (2022). Impact of urbanization on soil microbial diversity and composition in the megacity of Shanghai. *Land Degradation & Development*, *33*(2), 282-293.

Liu, Y., Huang, Q., Hu, W., Qin, J., Zheng, Y., Wang, J., Wang, Q., Xu, Y., Guo, G., Hu, S., & Xu, L. (2021). Effects of plastic mulch film residues on soil-microbe-plant systems under different soil pH conditions. *Chemosphere*, *267*, 128901.

Loewenberg, J. R., Sullivan, T., & Schuster, M. L. (1960). The effect of pH and minerals on the hatching and survival of Meloidogyne incognita larvae.

Lokatis, S., & Jeschke, J. M. (2022). Urban biotic homogenization: Approaches and knowledge gaps. *Ecological Applications*, *32*(8), e2703.

Macfadyen, A. (1961). Improved funnel-type extractors for soil arthropods. *The Journal of Animal Ecology*, 171-184.

Machado, J. D. S., Oliveira Filho, L. C. I., Santos, J. C. P., Paulino, A. T., & Baretta, D. (2019). Morphological diversity of springtails (Hexapoda: Collembola) as soil quality bioindicators in land use systems. *Biota Neotropica*, *19*(1), e20180618.

Magle, S. B., Theobald, D. M., & Crooks, K. R. (2009). A comparison of metrics predicting landscape connectivity for a highly interactive species along an urban gradient in Colorado, USA. *Landscape ecology*, *24*, 267-280.

Makarova, O. L., & Kolesnikova, A. A. (2019). Earthworms (Oligochaeta, Lumbricidae) in the tundra of Eastern Europe. *Biology Bulletin*, *46*, 438-449.

Manu, M., Băncilă, R. I., Mountford, O. J., Maruşca, T., Blaj, V. A., & Onete, M. (2022). Soil mite (Acari: Mesostigmata) communities and their relationships with some environmental variables in experimental grasslands from Bucegi Mountains in Romania. *Insects*, *13*(3), 285.

Manu, M., Honciuc, V., Neagoe, A., Băncilă, R. I., Iordache, V., & Onete, M. (2019). Soil mite communities (Acari: Mesostigmata, Oribatida) as bioindicators for environmental conditions from polluted soils. *Scientific reports*, *9*(1), 20250.

Manu, M., Onete, M., & Băncilă, R. I. (2018). The effect of heavy metals on mite communities (Acari: Gamasina) from urban parks-Bucharest, Romania. *Environmental Engineering & Management Journal (EEMJ)*, *17*(9).

Manu, M., Onete, M., Florescu, L., Bodescu, F., & Iordache, V. (2017). Influence of heavy metal pollution on soil mite communities (Acari) in Romanian grasslands. *North-Western Journal of Zoology*, *13*(2).

Martinson, H. M., & Raupp, M. J. (2013). A meta-analysis of the effects of urbanization on ground beetle communities. *Ecosphere*, *4*(5), 1-24.

McDonnell, M. J., & Hahs, A. K. (2008). The use of gradient analysis studies in advancing our understanding of the ecology of urbanizing landscapes: current status and future directions. *Landscape Ecology*, *23*, 1143-1155.

McFrederick, Q. S., & LeBuhn, G. (2006). Are urban parks refuges for bumble bees Bombus spp.(Hymenoptera: Apidae)?. *Biological conservation*, *129*(3), 372-382.

McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological conservation*, *127*(3), 247-260.

Melakeberhan, H., Dey, J., Baligar, V. C., & Carter Jr, T. E. (2004). Effect of soil pH on the pathogenesis of Heterodera glycines and Meloidogyne incognita on Glycine max genotypes. *Nematology*, *6*(4), 585-592.

Menta, C., Leoni, A., Bardini, M., Gardi, C., & Gatti, F. (2008). Nematode and microarthropod communities: comparative use of soil quality bioindicators in covered dump and natural soils. *Environmental Bioindicators*, *3*(1), 35-46.

Migliorini, M., Pigino, G., Bianchi, N., Bernini, F., & Leonzio, C. (2004). The effects of heavy metal contamination on the soil arthropod community of a shooting range. *Environmental Pollution*, *129*(2), 331-340.

Milano, V., Maisto, G., Baldantoni, D., Bellino, A., Bernard, C., Croce, A., Dubs, F., Strumia, S., & Cortet, J. (2018). The effect of urban park landscapes on soil Collembola diversity: A Mediterranean case study. *Landscape and Urban Planning*, *180*, 135-147.

Milleret, R., Le Bayon, R. C., & Gobat, J. M. (2009). Root, mycorrhiza and earthworm interactions: their effects on soil structuring processes, plant and soil nutrient concentration and plant biomass. *Plant and soil*, *316*, 1-12.

Nassauer, J. I., & Raskin, J. (2014). Urban vacancy and land use legacies: A frontier for urban ecological research, design, and planning. *Landscape and urban planning*, *125*, 245-253.

Neher, D. A. (2010). Ecology of plant and free-living nematodes in natural and agricultural soil. *Annual review of phytopathology*, *48*(1), 371-394.

Neher, D. A., Wu, J., Barbercheck, M. E., & Anas, O. (2005). Ecosystem type affects interpretation of soil nematode community measures. *Applied Soil Ecology*, *30*(1), 47-64.

Neilson, R., Caul, S., Fraser, F. C., King, D., Mitchell, S. M., Roberts, D. M., & Giles, M. E. (2020). Microbial community size is a potential predictor of nematode functional group in limed grasslands. *Applied Soil Ecology*, *156*, 103702.

Nielsen, A. B., Van Den Bosch, M., Maruthaveeran, S., & van den Bosch, C. K. (2014). Species richness in urban parks and its drivers: A review of empirical evidence. *Urban ecosystems*, *17*, 305-327.

Niemelä, J., & Kotze, D. J. (2009). Carabid beetle assemblages along urban to rural gradients: A review. *Landscape and Urban Planning*, *92*(2), 65-71.

Noronha, M. D. A., Fernandes, M. F., Maria de Fatima, S. M., Pedrosa, E. M. R., Assunção, M. C., & Calheiros, L. C. D. S. (2020). Soil abiotic factors associated with Meloidogyne spp. and Pratylenchus spp. populations in sugarcane. *Nematology*, *23*(2), 125-137.

Nunes dos Santos, P. S. (2008). Utilização de Colêmbolos edáficos (Insecta: Collembola) como indicadores da qualidade do solo, com recurso a características funcionais. *Departamento de Zoologia, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Mestrado em Ecologia, Coimbra*.

Nuutinen, V. (1992). Earthworm community response to tillage and residue management on different soil types in southern Finland. *Soil and Tillage Research*, *23*(3), 221-239.

Oliveira Filho, L. C. I., & Baretta, D. (2016). Por que devemos nos importar com os colêmbolos edáficos?. *Scientia agraria*, *17*(2), 21-40.

Otoshi, M. D., Bichier, P., & Philpott, S. M. (2015). Local and landscape correlates of spider activity density and species richness in urban gardens. *Environmental Entomology*, *44*(4), 1043-1051.

Pagotto, C., Remy, N., Legret, M., & Le Cloirec, P. (2001). Heavy metal pollution of road dust and roadside soil near a major rural highway. *Environmental technology*, *22*(3), 307-319.

Pahalvi, H. N., Rafiya, L., Rashid, S., Nisar, B., & Kamili, A. N. (2021). Chemical fertilizers and their impact on soil health. *Microbiota and Biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs*, 1-20.

Pantaloni, M., Marinelli, G., Santilocchi, R., Minelli, A., & Neri, D. (2022). Sustainable management practices for urban green spaces to support green infrastructure: an italian case study. *Sustainability*, *14*(7), 4243.

Parisi, V. (2001). La qualità biologica del suolo. Un metodo basato sui microartropodi. *Acta Naturalia de L'Ateneo Parmense*, *37*, 105-114.

Parisi, V., Menta, C., Gardi, C., Jacomini, C., & Mozzanica, E. (2005). Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agriculture, ecosystems & environment*, *105*(1-2), 323-333.

Parris, K. M. (2006). Urban amphibian assemblages as metacommunities. *Journal of Animal Ecology*, *75*(3), 757-764.

Pavao-Zuckerman, M. A., & Coleman, D. C. (2007). Urbanization alters the functional composition, but not taxonomic diversity, of the soil nematode community. *Applied Soil Ecology*, *35*(2), 329-339.

Peng, J., Zhao, H., & Liu, Y. (2017). Urban ecological corridors construction: A review. *Acta Ecologica Sinica*, *37*(1), 23-30.

Pen-Mouratov, S., Shukurov, N., & Steinberger, Y. (2008). Influence of industrial heavy metal pollution on soil free-living nematode population. *Environmental pollution*, *152*(1), 172-183.

Perry, K. I., Hoekstra, N. C., Delgado de la flor, Y. A., & Gardiner, M. M. (2020). Disentangling landscape and local drivers of ground-dwelling beetle community assembly in an urban ecosystem. *Ecological Applications*, *30*(8), e02191.

Perry, R. N., Moens, M., & Jones, J. T. (Eds.). (2024). *Plant nematology*. Cabi.

Petersen, H., & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos*, 288-388.

Piano, E., Bona, F., & Isaia, M. (2020). Urbanization drivers differentially affect ground arthropod assemblages in the city of Turin (NW-Italy). *Urban Ecosystems*, *23*(3), 617-629.

Piotrowska, K., Connolly, J., Finn, J., Black, A., & Bolger, T. (2013). Evenness and plant species identity affect earthworm diversity and community structure in grassland soils. *Soil Biology and Biochemistry*, *57*, 713-719.

Plum, N. M., & Filser, J. (2005). Floods and drought: Response of earthworms and potworms (Oligochaeta: Lumbricidae, Enchytraeidae) to hydrological extremes in wet grassland. *Pedobiologia*, *49*(5), 443-453.

Ponge, J. F., Dubs, F., Gillet, S., Sousa, J. P., & Lavelle, P. (2006). Decreased biodiversity in soil springtail communities: the importance of dispersal and landuse history in heterogeneous landscapes. *Soil Biology and Biochemistry*, *38*(5), 1158-1161.

Ponge, J. F., Gillet, S., Dubs, F., Fedoroff, E., Haese, L., Sousa, J. P., & Lavelle, P. (2003). Collembolan communities as bioindicators of land use intensification. *Soil biology and biochemistry*, *35*(6), 813-826.

Pouyat, R. V., Yesilonis, I. D., Dombos, M., Szlavecz, K., Setälä, H., Cilliers, S., Hornung, E., Kotze, D. J., & Yarwood, S. (2015). A global comparison of surface soil characteristics across five cities: a test of the urban ecosystem convergence hypothesis. *Soil Science*, *180*(4/5), 136-145.

Pukowiec-Kurda, K. (2022). The urban ecosystem services index as a new indicator for sustainable urban planning and human well-being in cities. *Ecological Indicators*, *144*, 109532.

Pukowiec-Kurda, K., & Vavrouchová, H. (2020). Land cover change and landscape transformations (2000–2018) in the rural municipalities of the upper Silesia-Zagłębie metropolis. *Sustainability*, *12*(23), 9911.

Raczuk, J., & Pokora, A. (2008). Influence of traffic pollution on heavy metals content in soil and earthworms (Lumbricidae). *Ecological Chemistry and Engineering*, *15*, 265-274.

Reese, A. T., Savage, A., Youngsteadt, E., McGuire, K. L., Koling, A., Watkins, O., Frank, S. D., & Dunn, R. R. (2016). Urban stress is associated with variation in microbial species composition—but not richness—in Manhattan. *The ISME journal*, *10*(3), 751-760.

Reis, F., Carvalho, F., da Silva, P. M., Mendes, S., Santos, S. A., & Sousa, J. P. (2016). The use of a functional approach as surrogate of Collembola species richness in European perennial crops and forests. *Ecological Indicators*, *61*, 676-682.

Renaud, A., Poinsot-Balaguer, N., Cortet, J., & Le Petit, J. (2004). Influence of four soil maintenance practices on Collembola communities in a Mediterranean vineyard. *Pedobiologia*, *48*(5-6), 623-630.

Richardson, D. R., Snyder, B. A., & Hendrix, P. F. (2009). Soil moisture and temperature: tolerances and optima for a non-native earthworm species, Amynthas agrestis (Oligochaeta: Opisthopora: Megascolecidae). *Southeastern Naturalist*, *8*(2), 325-334.

Richter, A., Kern, T., Wolf, S., Struck, U., & Ruess, L. (2019). Trophic and non-trophic interactions in binary links affect carbon flow in the soil micro-food web. *Soil Biology and Biochemistry*, *135*, 239-247.

Ricketts, T. H. (2001). The matrix matters: effective isolation in fragmented landscapes. *The American Naturalist*, *158*(1), 87-99.

Römbke, J., Sousa, J. P., Schouten, T., & Riepert, F. (2006). Monitoring of soil organisms: a set of standardized field methods proposed by ISO. *European Journal of Soil Biology*, *42*, S61-S64.

Rook, G. A. (2013). Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. *Proceedings of the National Academy of Sciences*, *110*(46), 18360-18367.

Rosselló-Mora, R., & Amann, R. (2001). The species concept for prokaryotes. *FEMS microbiology reviews*, *25*(1), 39-67.

Ruess, L. (2003). Nematode soil faunal analysis of decomposition pathways in different ecosystems. *Nematology*, *5*(2), 179-181.

Rutgers, M., Orgiazzi, A., Gardi, C., Römbke, J., Jänsch, S., Keith, A. M., Neilson, R., Boag, B., Schmidt, O., Murchie, A. K., Blackshaw, R. P., Pérès, G., Cluzeau, D., Guernion, M., Briones, M. J. I., Rodeiro, J., Piñeiro, R., Díaz Cosín, D. J., Sousa, J. P., Suhadolc, M., & De Zwart, D. (2016). Mapping earthworm communities in Europe. *Applied Soil Ecology*, *97*, 98-111.

Rzeszowski, K., & Sterzyńska, M. (2016). Changes through time in soil Collembola communities exposed to urbanization. *Urban ecosystems*, *19*, 143-158.

Rzeszowski, K., Zadrożny, P., & Nicia, P. (2017). The effect of soil nutrient gradients on Collembola communities inhabiting typical urban green spaces. *Pedobiologia*, *64*, 15-24.

Sabais, A. C., Scheu, S., & Eisenhauer, N. (2011). Plant species richness drives the density and diversity of Collembola in temperate grassland. *Acta Oecologica*, *37*(3), 195-202.

Sadler, J. P., Small, E. C., Fiszpan, H., Telfer, M. G., & Niemelä, J. (2006). Investigating environmental variation and landscape characteristics of an urban–rural gradient using woodland carabid assemblages. *Journal of biogeography*, *33*(6), 1126-1138.

Salamon, J. A., Schaefer, M., Alphei, J., Schmid, B., & Scheu, S. (2004). Effects of plant diversity on Collembola in an experimental grassland ecosystem. *Oikos*, *106*(1), 51-60.

Šalamún, P., Kucanová, E., Brázová, T., Miklisová, D., Renčo, M., & Hanzelová, V. (2014). Diversity and food web structure of nematode communities under high soil salinity and alkaline pH. *Ecotoxicology*, *23*, 1367-1376.

Šalamún, P., Renčo, M., Kucanová, E., Brázová, T., Papajová, I., Miklisová, D., & Hanzelová, V. (2012). Nematodes as bioindicators of soil degradation due to heavy metals. *Ecotoxicology*, *21*, 2319-2330.

Sánchez-Moreno, S., & Ferris, H. (2018). Nematode ecology and soil health. In *Plant parasitic nematodes in subtropical and tropical agriculture* (pp. 62-86). Wallingford UK: CAB International.

Santos, M. A. B. D., Oliveira Filho, L. C. I. D., Pompeo, P. N., Ortiz, D. C., Mafra, Á. L., Klauberg Filho, O., & Baretta, D. (2018). Morphological diversity of springtails in land use systems. *Revista Brasileira de Ciência do Solo*, *42*, e0170277.

Schittko, C., Onandia, G., Bernard-Verdier, M., Heger, T., Jeschke, J. M., Kowarik, I., Maaß, S., & Joshi, J. (2022). Biodiversity maintains soil multifunctionality and soil organic carbon in novel urban ecosystems. *Journal of Ecology*, *110*(4), 916-934.

Schrama, M., De Haan, J. J., Kroonen, M., Verstegen, H., & Van der Putten, W. H. (2018). Crop yield gap and stability in organic and conventional farming systems. *Agriculture, ecosystems & environment*, *256*, 123-130.

Seastedt, T. R., & Crossley Jr, D. A. (1980). Effects of microarthropods on the seasonal dynamics of nutrients in forest litter. *Soil Biology and Biochemistry*, *12*(4), 337-342.

Setälä, H., Francini, G., Allen, J. A., Jumpponen, A., Hui, N., & Kotze, D. J. (2017). Urban parks provide ecosystem services by retaining metals and nutrients in soils. *Environmental Pollution*, *231*, 451-461.

Seto, K. C., Fragkias, M., Güneralp, B., & Reilly, M. K. (2011). A meta-analysis of global urban land expansion. *PloS one*, *6*(8), e23777.

Sharma, K., Cheng, Z., & Grewal, P. S. (2015). Relationship between soil heavy metal contamination and soil food web health in vacant lots slated for urban agriculture in two post-industrial cities. *Urban ecosystems*, *18*, 835-855.

Sherlock, E. (2012). *Key to the Earthworms of the UK and Ireland*. FSC.

Siebert, J., Ciobanu, M., Schädler, M., & Eisenhauer, N. (2020). Climate change and land use induce functional shifts in soil nematode communities. *Oecologia*, *192*(1), 281-294.

Sieriebriennikov, B., Ferris, H., & de Goede, R. G. (2014). NINJA: An automated calculation system for nematode-based biological monitoring. *European Journal of Soil Biology*, *61*, 90-93.

Sims, R. W., & Gerard, B. M. (2023). *Earthworms: keys and notes for the identification and study of the species* (Vol. 31). Brill.

Song, H., Singh, D., Tomlinson, K. W., Yang, X., Ogwu, M. C., Slik, J. F., & Adams, J. M. (2019). Tropical forest conversion to rubber plantation in southwest China results in lower fungal beta diversity and reduced network complexity. *FEMS microbiology ecology*, *95*(7), fiz092.

Srivastava, D. (2009). *Key to common soil mites*. Department of Zoology, University of British Columbia. https://www.zoology.ubc.ca/~srivast/mites/key.html

Stein, M. M., Hrusch, C. L., Gozdz, J., Igartua, C., Pivniouk, V., Murray, S. E., Ledford, J. G., dos Santos, M. M., Anderson, R. L., Metwali, N., Neilson, J. W., Maier, R. M., Gilbert, J. A., Holbreich, M., Thorne, P. S., Martinez, F. D., von Mutius, E., Vercelli, D., Ober, C., & Sperling, A. I. (2016). Innate immunity and asthma risk in Amish and Hutterite farm children. *New England journal of medicine*, *375*(5), 411-421.

Swift, M. J., Heal, O. W., Anderson, J. M., & Anderson, J. M. (1979). *Decomposition in terrestrial ecosystems* (Vol. 5). Univ of California Press.

Sylvain, Z. A., Wall, D. H., Cherwin, K. L., Peters, D. P., Reichmann, L. G., & Sala, O. E. (2014). Soil animal responses to moisture availability are largely scale, not ecosystem dependent: insight from a cross-site study. *Global Change Biology*, *20*(8), 2631-2643.

Szabó, B., Korányi, D., Gallé, R., Lövei, G. L., Bakonyi, G., & Batáry, P. (2023). Urbanization decreases species richness, and increases abundance in dry climates whereas decreases in wet climates: a global meta-analysis. *Science of the Total Environment*, *859*, 160145.

Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B., & Buchmann, N. (2007). Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia*, *151*, 190-205.

Ter Braak, C. J., & Verdonschot, P. F. (1995). Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic sciences*, *57*, 255-289.

Terhivuo, J., & Valovirta, I. (1978). Habitat spectra of the Lumbricidae (Oligochaeta) in Finland. In *Annales Zoologici Fennici* (pp. 202-209). Finnish Academy of Sciences, Societas Scientiarum Fennica, Societas pro Fauna et Flora Fennica and Societas Biologica Fennica Vanamo.

Testerink, G. J. (1983). Metabolic adaptations to seasonal changes in humidity and temperature in litter-inhabiting Collembola. *Oikos*, 234-240.

Thornton, C. W., & Matlack, G. R. (2002). Long-term disturbance effects in the nematode communities of south Mississippi woodlands. *Journal of Nematology*, *34*(2), 88.

Timper, P., Davis, R., Jagdale, G., & Herbert, J. (2012). Resiliency of a nematode community and suppressive service to tillage and nematicide application. *Applied Soil Ecology*, *59*, 48-59.

Tóth, Z., Szlavecz, K., Schmidt, D. J. E., Hornung, E., Setälä, H., Yesilonis, I. D., Kotze, D. J., Dombos, M., Pouyat, R., Mishra, S., Cilliers, S., Yarwood, S., & Csuzdi, C. (2020). Earthworm assemblages in urban habitats across biogeographical regions. *Applied Soil Ecology*, *151*, 103530.

Tresch, S., Frey, D., Le Bayon, R. C., Zanetta, A., Rasche, F., Fliessbach, A., & Moretti, M. (2019). Litter decomposition driven by soil fauna, plant diversity and soil management in urban gardens. *Science of the Total Environment*, *658*, 1614-1629.

United Nations Department of Economic and Social Affairs. (2018). World Population Projected to Live in Urban Areas by 2050, Says UN. Retrieved from https://www.un.org/en/desa/68-world-population-projected-live-urban-areas-2050-says-un

Valchovski, H. (2010). Characteristic of population of earthworm family Lumbricidae in parks of Sofia as bioindicator of soil health.

Valtanen, M., Sillanpää, N., & Setälä, H. (2015). Key factors affecting urban runoff pollution under cold climatic conditions. *Journal of Hydrology*, *529*, 1578-1589.

Van Den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D. A., de Goede, R. G. M., Adams, B. J., Ahmad, W., Andriuzzi, W. S., Bardgett, R. D., Bonkowski, M., Campos-Herrera, R., Cares, J. E., Caruso, T., Caixeta, L. B., Chen, X., Costa, S. R., Creamer, R., Castro, J. M. da C., Dam, M., Djigal, D., Escuer, M., Griffiths, B. S., Gutiérrez, C., Hohberg, K., Kalinkina, D., Kardol, P., Kergunteuil, A., Korthals, G., Krashevska, V., Kudrin, A. A., Li, Q., Liang, W., Magilton, M., Marais, M., Martín, J. A. R., Matveeva, E., Mayad, E. H., Mulder, C., Mullin, P., Neilson, R., Nguyen, T. A. D., Nielsen, U. N., Okada, H., Palomares Rius, J. E., Pan, K., Peneva, V., Pellissier, L., Pereira da Silva, J. C., Pitteloud, C., Powers, T. O., Powers, K., Quist, C. W., Rasmann, S., Sánchez Moreno, S., Scheu, S., Setälä, H., Sushchuk, A., Tiunov, A. V., Trap, J., van der Putten, W., Vestergård, M., Villenave, C., Waeyenberge, L., Wall, D. H., Wilschut, R., Wright, D. G., Yang, J.I., & Crowther, T. W. (2019). Soil nematode abundance and functional group composition at a global scale. *Nature*, *572*(7768), 194-198.

Vandewalle, M., De Bello, F., Berg, M. P., Bolger, T., Dolédec, S., Dubs, F., Feld, C. K., Harrington, R., Harrison, P. A., Lavorel, S., Martins da Silva, P., Moretti, M., Niemelä, J., Santos, P., Sattler, T., Sousa, J. P., Sykes, M. T., Vanbergen, A. J., & Woodcock, B. A. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. *Biodiversity and Conservation*, *19*, 2921-2947.

Wagg, C., Bender, S. F., Widmer, F., & Van Der Heijden, M. G. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, *111*(14), 5266-5270.

Wall, D. H., Bardgett, R. D., Behan-Pelletier, V., Herrick, J. E., Jones, T. H., Six, J., Strong, D. R., van der Putten, W. H., & Ritz, K. (Eds.). (2012). *Soil ecology and ecosystem services*. OUP Oxford.

Wall, D. H., Nielsen, U. N., & Six, J. (2015). Soil biodiversity and human health. *Nature*, *528*(7580), 69-76.

Walworth, J. L. (2011). Soil sampling and analysis.

Wang, C., Zhao, C., Xu, Z., Wang, Y., & Peng, H. (2013). Effect of vegetation on soil water retention and storage in a semi-arid alpine forest catchment. *Journal of arid land*, *5*, 207-219.

Wang, H., Marshall, C. W., Cheng, M., Xu, H., Li, H., Yang, X., & Zheng, T. (2017). Changes in land use driven by urbanization impact nitrogen cycling and the microbial community composition in soils. *Scientific reports*, *7*(1), 44049.

Wang, M., & Zhang, H. (2018). Accumulation of heavy metals in roadside soil in urban area and the related impacting factors. *International journal of environmental research and public health*, *15*(6), 1064.

Whitehead, A. G., & Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of applied Biology*, *55*(1), 25-38.

Wilke, B. M. (2005). Determination of chemical and physical soil properties. *Monitoring and assessing soil bioremediation*, 47-95.

Wissuwa, J., Salamon, J. A., & Frank, T. (2012). Effects of habitat age and plant species on predatory mites (Acari, Mesostigmata) in grassy arable fallows in Eastern Austria. *Soil Biology and Biochemistry*, *50*, 96-107.

Wurst, S., De Deyn, G. B., & Orwin, K. (2012). Soil biodiversity and functions. *Soil ecology and ecosystem services*, 28-44.

Xie, T., Li, X., Wang, M., Chen, W., & Faber, J. H. (2022). Factors Influencing Earthworm Fauna in Parks in Megacity Beijing, China: An Application of a Synthetic and Simple Index (ESI). *Sustainability*, *14*(10), 6054.

Xie, T., Wang, M., Chen, W., & Uwizeyimana, H. (2018). Impacts of urbanization and landscape patterns on the earthworm communities in residential areas in Beijing. *Science of the Total Environment*, *626*, 1261-1269.

Yeates, G. W. (2007). Abundance, diversity, and resilience of nematode assemblages in forest soils. *Canadian journal of forest research*, *37*(2), 216-225.

Yeates, G. W., & Bongers, T. (1999). Nematode diversity in agroecosystems. In *Invertebrate biodiversity as bioindicators of sustainable landscapes* (pp. 113-135). Elsevier.

Yeates, G. W., Bongers, T., De Goede, R. G., Freckman, D. W., & Georgieva, S. S. (1993). Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of nematology*, *25*(3), 315.

Yeates, G. W., Ferris, H., Moens, T., & Putten, W. V. D. (2009). The role of nematodes in ecosystems. In *Nematodes as environmental indicators* (pp. 1-44). Wallingford UK: CABI.

Yu, H., Ding, W., Luo, J., Geng, R., & Cai, Z. (2012). Long-term application of organic manure and mineral fertilizers on aggregation and aggregate-associated carbon in a sandy loam soil. *Soil and Tillage Research*, *124*, 170-177.

Yu, S., Qiu, J., Chen, X., Luo, X., Yang, X., Wang, F., & Xu, G. (2021). Soil mesofauna community changes in response to the environmental gradients of urbanization in Guangzhou city. *Frontiers in Ecology and Evolution*, *8*, 546433.

Zhang, X., Guan, P., Wang, Y., Li, Q., Zhang, S., Zhang, Z., Bezemer, T. M., & Liang, W. (2015). Community composition, diversity and metabolic footprints of soil nematodes in differently-aged temperate forests. *Soil biology and biochemistry*, *80*, 118-126.

Zhao, J., & Neher, D. A. (2013). Soil nematode genera that predict specific types of disturbance. *Applied Soil Ecology*, *64*, 135-141.

Zhao, J., & Neher, D. A. (2014). Soil energy pathways of different ecosystems using nematode trophic group analysis: a meta analysis. *Nematology*, *16*(4), 379-385.

Zhao, J., Zhang, W., Liu, X., Yang, R., Xiao, D., He, X., & Wang, K. (2023). Grass harvesting eliminates the beneficial effects of legume addition on soil nematode communities in a tall grass pasture. *Agriculture, Ecosystems & Environment*, *349*, 108468.

Ziter, C. (2016). The biodiversity–ecosystem service relationship in urban areas: a quantitative review. *Oikos*, *125*(6), 761-768.

6. Annexes

Annex 1. Mean total number and respective standard errors of nematode individuals per family/genera obtained from soil samples across different land use types in the city of Braga.

Nematode Family/Genera	Unused lands	Lawns	Flower beds	Forests	Roadsides
Fungivores					
Aphelenchoididae	519.5 ± 220.1	1058.2 ± 174	164.4 ± 50.2	810.8 ± 178.9	544.5 ± 102.2
Aphelenchidae	42.4 ± 17.9	58.9 ± 19	45.9 ± 23	11.2 ± 5.6	151.8 ± 56.7
Diphtherophoridae	5.7 ± 4.2	14.7 ± 6.9	23.2 ± 12.7	15.6 ± 4.6	10.8 ± 6.7
Tylencholaimus	3 ± 1.6	2.2 ± 2.2	0 ± 0	54.8 ± 30.5	0 ± 0
Bacterivores					
Panagrolaimidae	151.6 ± 90.9	1163.3 ± 564.4	538.8 ± 263.3	205.4 ± 48.9	585.2 ± 472.8
Cephalobidae	281 ± 148.8	824.7 ± 353.8	600.5 ± 146.2	299 ± 79.2	537.8 ± 193.9
Rhabditidae	8.7 ± 8.7	128.6 ± 54.5	227.6 ± 64.5	20.1 ± 7.8	22.1 ± 13.3
Teratocephalidae	19.3 ± 12.7	19.2 ± 8.9	43.6 ± 23	175.7 ± 43.3	23.1 ± 18.6
Prismatolaimidae	48.4 ± 18.1	77.8 ± 12.3	224.4 ± 78.8	82.7 ± 21.4	80.3 ± 37.1
Wilsonema	29.9 ± 20.5	0 ± 0	33.9 ± 30.5	213.3 ± 57.7	1.3 ± 1.3
Plectidae	4.3 ± 4.3	0 ± 0	2.9 ± 2.9	1.2 ± 1.2	0 ± 0
Alaimidae	12.6 ± 7.8	0 ± 0	31.9 ± 3.6	39.6 ± 18.1	12.7 ± 12.7
Herbivores					
Helicotylenchus	49.1 ± 37.3	126.3 ± 97.9	126.8 ± 59	60.5 ± 39.4	50 ± 27.7
Rotylenchus	1.3 ± 1.3	12.2 ± 12.2	22.6 ± 15.4	4.8 ± 4.8	0 ± 0
Paratylenchus	113.9 ± 39.7	15.7 ± 15.7	5.8 ± 5.8	43 ± 25.1	12.6 ± 9.8
Pratylenchus	97.2 ± 74.2	40.5 ± 14.6	77.9 ± 34.2	9.2 ± 9.2	205.6 ± 124.1
Meloidogyne	41.1 ± 35.4	48.5 ± 38.6	186.8 ± 59.2	0 ± 0	30.6 ± 12.1
Tylenchorhynchus	72.2 ± 41	7.5 ± 7.5	4.2 ± 4.2	0 ± 0	10.1 ± 10.1
Hoplolaimus	12.7 ± 8.6	0 ± 0	0 ± 0	0 ± 0	3.5 ± 2.5
Xiphinema	0 ± 0	2.2 ± 2.2	5.7 ± 3.5	24.5 ± 24.5	0 ± 0
Gracilacus	4.3 ± 4.3	0 ± 0	0 ± 0	24.5 ± 15	0 ± 0
Criconematidae	0 ± 0	0 ± 0	18.9 ± 12.6	18.4 ± 12.3	0 ± 0
Cacopaurus	1.5 ± 1.5	0 ± 0	0 ± 0	4.1 ± 4.1	0 ± 0
Hemicycliophora	0 ± 0	0 ± 0	7 ± 4.5	0 ± 0	0 ± 0
Trichodoridae	4.3 ± 4.3	0 ± 0	18.3 ± 6	0 ± 0	3.3 ± 2.1
Predators					
Qudsianematidae	1.4 ± 1.4	2.2 ± 2.2	0 ± 0	0 ± 0	0 ± 0
Aporcelaimidae	51.7 ± 41.6	21 ± 9.6	7.3 ± 4.6	34.6 ± 11.6	38.1 ± 21.2

Dorylaimidae	5.4 ± 5.4	7.2 ± 7.2	18.7 ± 6.9	4.8 ± 3	0 ± 0
Omnivores					
Mononchidae	4.8 ± 4.2	37.7 ± 11.8	25.4 ± 7.3	33.1 ± 13.7	7.7 ± 5.1
Tripylidae	0 ± 0	0 ± 0	2.1 ± 2.1	9.4 ± 7.9	0 ± 0

Annex 2. Total of morphotypes and corresponding EMI scores, as well as partial EMI scores for each characteristic of Collembolans obtained from soil samples across different land use types in the city of Braga.

	Ocelli	**Antenna size**	**Furcula**	**Hairs/Scales**	**Pigmentation**	**EMI**
edap1	4	4	4	0	4	16
edap2	4	4	2	0	4	14
edap3	4	4	4	0	2	14
edap4	4	4	2	0	2	12
edap5	0	4	4	0	4	12
semied1	0	4	4	0	2	10
semied2	0	4	2	0	4	10
semied3	0	4	2	0	2	8
semied4	0	2	2	0	4	8
epig1	0	4	0	0	2	6
epig2	0	2	2	0	2	6
epig3	0	0	2	0	4	6
epig4	0	2	0	0	2	4
epig5	0	0	2	0	2	4

Annex 3. Mean total number and respective standard errors of Collembolan individuals per morphotype obtained from soil samples across different land use types in the city of Braga.

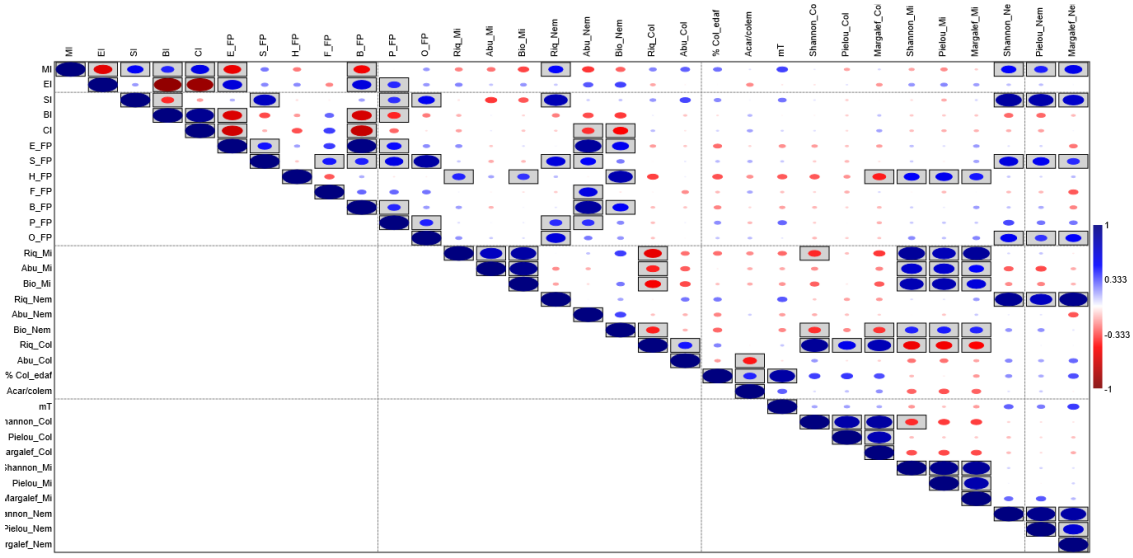
Collembola Morphotype	Unused lands	Lawns	Flower beds	Forests	Roadsides
edap 1	0.2 ± 0.2	2.4 ± 1.36	22.2 ± 12.44	16.8 ± 3.38	1.4 ± 1.17
edap 2	2.8 ± 1.66	1 ± 0.77	0.4 ± 0.4	5.8 ± 0.97	2.4 ± 1.12
edap 3	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0
edap 4	1.6 ± 1.36	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.4
edap 5	0 ± 0	0 ± 0	0 ± 0	0.6 ± 0.24	0 ± 0
semied1	0.2 ± 0.2	0 ± 0	2.2 ± 1.56	6.4 ± 4.23	0 ± 0
semied2	0 ± 0	0 ± 0	3.8 ± 3.8	1.8 ± 0.2	6 ± 1.52
semied3	8 ± 5.81	5.6 ± 2.69	13.8 ± 6.45	23.6 ± 16.21	32.2 ± 20.27
semied4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.2
epig1	0 ± 0	0.4 ± 0.4	0.8 ± 0.8	0 ± 0	0 ± 0
epig2	0.2 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
epig3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0.4
epig4	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0
epig5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.8 ± 0.8

Annex 4. Abundance of Mites obtained from soil samples across different land use types in the city of Braga.

Land use	Mites abundance
Unused lands	31.6 ± 9.8
Lawns	7.4 ± 3.7
Flower beds	15.4 ± 10.1
Forests	91.2 ± 27.7
Roadsides	6.6 ± 1.4

Annex 5. Mean total number and respective standard errors of earthworm individuals per species obtained from soil samples across different land use types in the city of Braga.

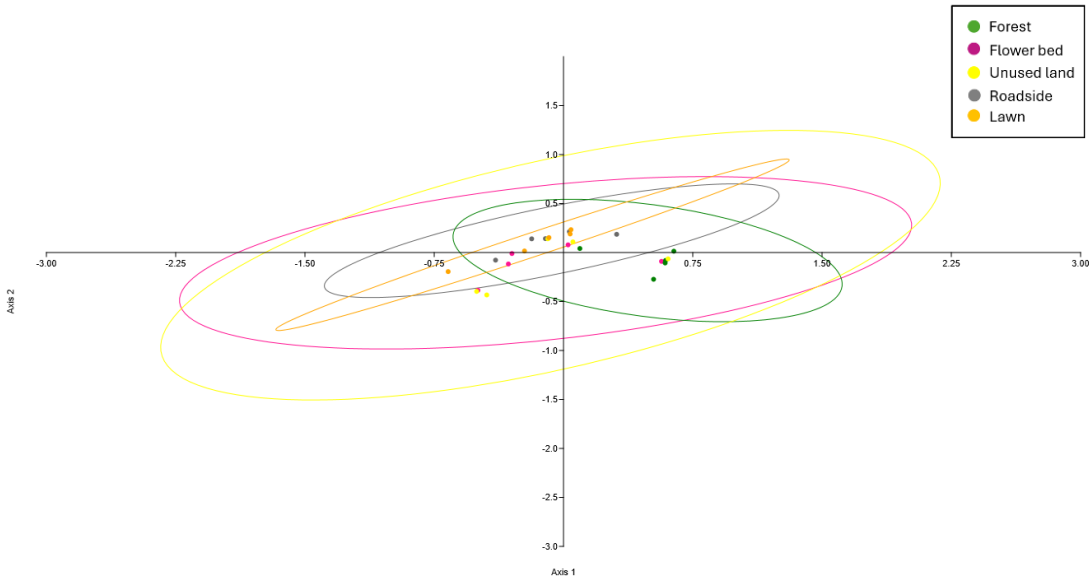
Earthworm specie	Unused lands	Lawns	Flower beds	Forests	Roadsides
Bimastos parvus	0 ± 0	0 ± 0	0.6 ± 0.6	0 ± 0	0 ± 0
Octolasion tyrtaeum	0 ± 0	0 ± 0	0 ± 0	12.2 ± 12.2	0 ± 0
Octolasion cyaneum	0 ± 0	0.4 ± 0.4	0 ± 0	0 ± 0	0.2 ± 0.2
Octolasion cyaneumlacteum	0.2 ± 0.2	0 ± 0	2 ± 2	0 ± 0	0 ± 0
Dendrobaena octaedra	0 ± 0	0 ± 0	0 ± 0	6.6 ± 5.65	0 ± 0
Dendrobaena hortensis	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dendrobaena rubidus	0 ± 0	0 ± 0	0.6 ± 0.6	0 ± 0	0 ± 0
Satchellius mammalis	0 ± 0	1.4 ± 1.4	0 ± 0	0.2 ± 0.2	0.4 ± 0.4
Aporrectodea chlorotica	6 ± 3.73	25 ± 11.04	2.2 ± 1.96	0 ± 0	11.2 ± 7.11
Aporrectodea caliginosa	0.6 ± 0.6	0.4 ± 0.24	0 ± 0	0 ± 0	6.2 ± 4.34
Aporrectodea longa	10.2 ± 7.32	13.6 ± 7.76	0.2 ± 0.2	0 ± 0	6.4 ± 6.15
Aporrectodea rosea	0.4 ± 0.4	2.2 ± 2.2	1.2 ± 1.2	0 ± 0	0 ± 0
Aporrectodea trapezoides	0.8 ± 0.8	1.8 ± 1.8	0 ± 0	0 ± 0	0 ± 0
Lumbricidae	0 ± 0	5 ± 5	0 ± 0	0 ± 0	1.8 ± 1.8
Microscolex dubius	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0
Microscolex phosphoreus	0 ± 0	0.8 ± 0.8	0.4 ± 0.24	0 ± 0	20 ± 19.5
Amynthas corticis	0 ± 0	0 ± 0	3.4 ± 2.09	0 ± 0	0 ± 0
Octodrilus sp.	0 ± 0	0.6 ± 0.6	0 ± 0	0 ± 0	0 ± 0
Juveniles	30.8 ± 10.63	14.2 ± 4.13	2.6 ± 2.14	1.8 ± 1.8	22.2 ± 8.28



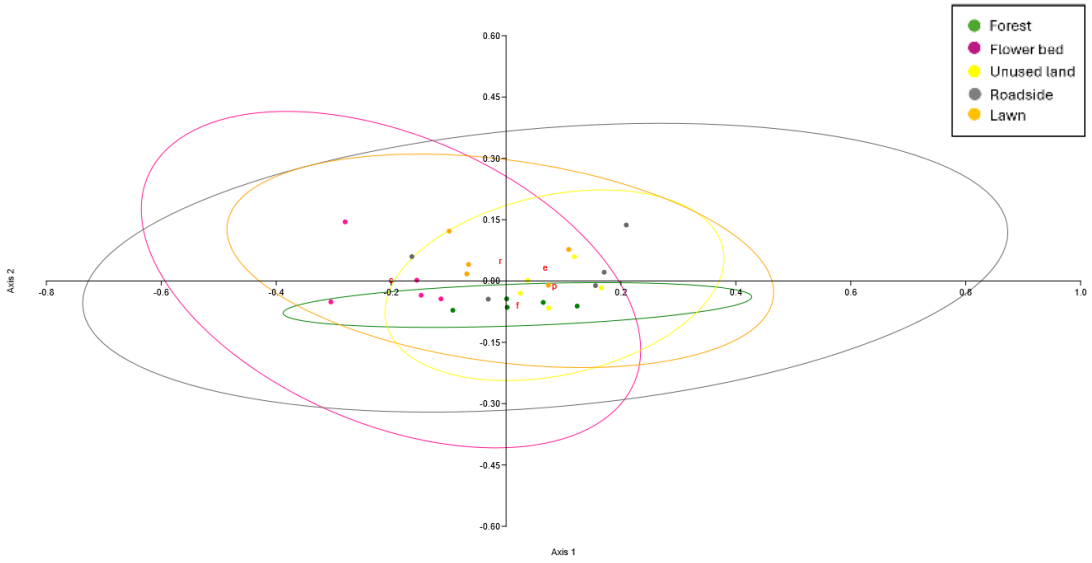
Annex 6. Pearson correlations of the Metrics calculated from the different communities of faunal groups. The circles represented as blue, correspond to positive correlations, whereas the circles represented as red, correspond to negative correlations. Significant correlations are delimited in squares ($p<0.05$).

Annex 7. Mean values and respective standard errors of physicochemical parameters measured in soil samples from different land use types in the city of Braga.

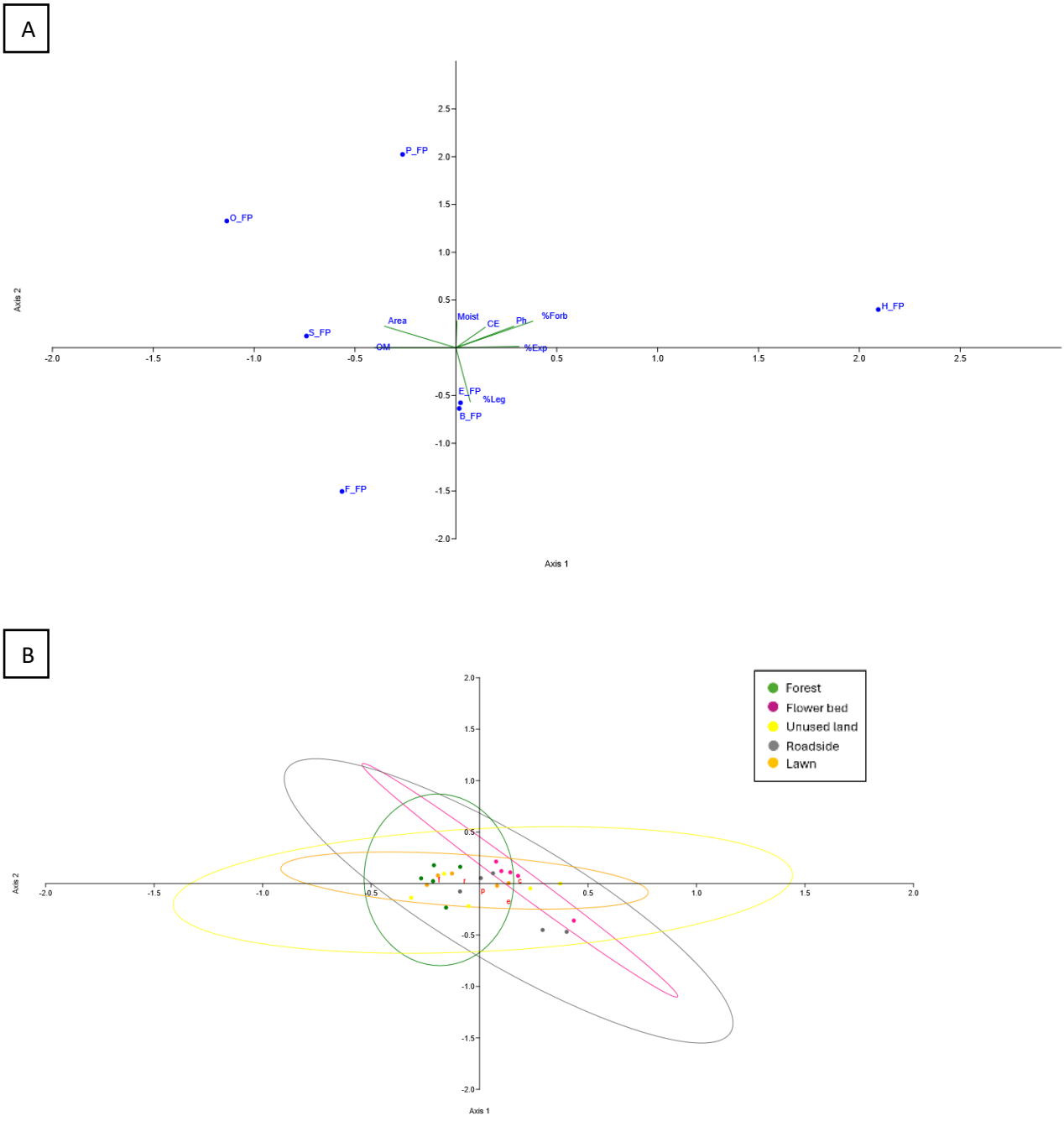
Land-use Type	Moisture %	Organic Matter %	pH	Electrical Conductivity (µS/cm)
Unused lands	22.62 ± 1.57	9.76 ± 1.97	5.62 ± 0.2	40.76 ± 7.07
Lawns	21.33 ± 1.09	7.11 ± 0.47	5.98 ± 0.24	76.5 ± 12.89
Flower beds	25.68 ± 1.36	9.81 ± 0.71	6.24 ± 0.26	81.66 ± 16.86
Forests	21.18 ± 2.21	15 ± 2.2	5 ± 0.19	51.64 ± 5.33
Roadsides	19.63 ± 1.19	7.75 ± 0.89	5.53 ± 0.35	66.74 ± 14.48



Annex 8. Canonical correspondence analysis of the influence of the environmental variables on nematodes, earthworms and collembolans biodiversity indices. Eigenvalues were 0.08516 and 0.01082 for the first and the second axis, respectively. Overlapping of 95% ellipses for the land use groups.

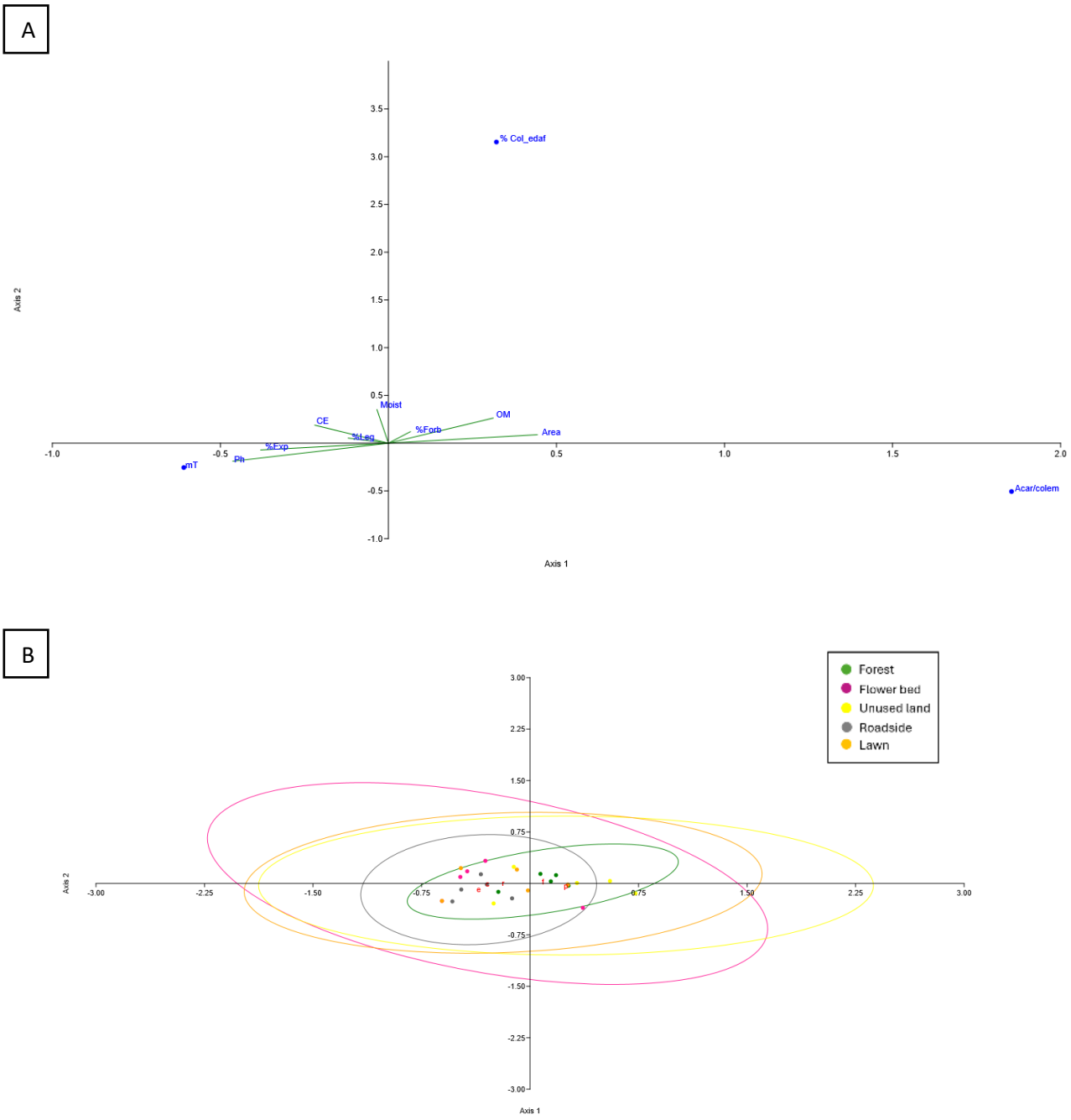


Annex 9. Canonical correspondence analysis of the influence of the environmental variables on nematode community indices. Eigenvalues were 0.0121 and 0.001593 for the first and the second axis, respectively. Overlapping of 95% ellipses for the land use groups.



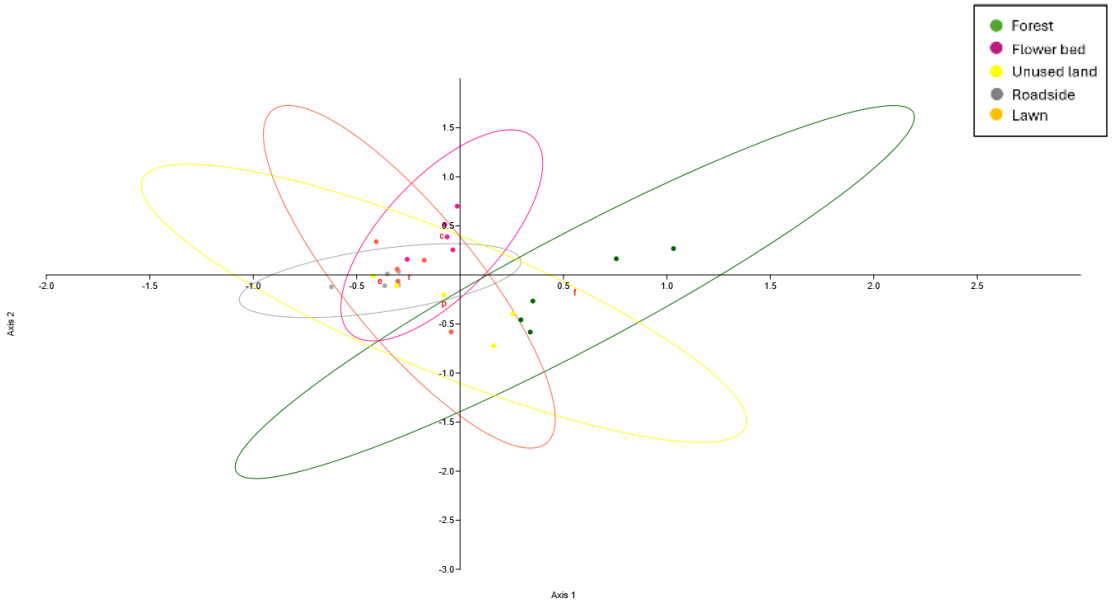
Annex 10. Canonical correspondence analysis of the influence of the environmental variables on nematode metabolic footprints. Eigenvalues were 0.02063 and 0.0091 for the first and the second axis, respectively. A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and the Enrichment, Structure, Bacterivore, Fungivore, Herbivore, Omnivore, and Predator Footprints (Triplot amplitude: x1). (B) overlapping of 95% ellipses for the land use groups. E_FP: Enrichment Footprint; S_FP: Structure Footprint; B_FP: Bacterivore Footprint; F_FP: Fungivore Footprint; H_FP: Herbivore Footprint; O_FP: Omnivore Footprint; P_FP: Predator Footprint; OM:

Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.

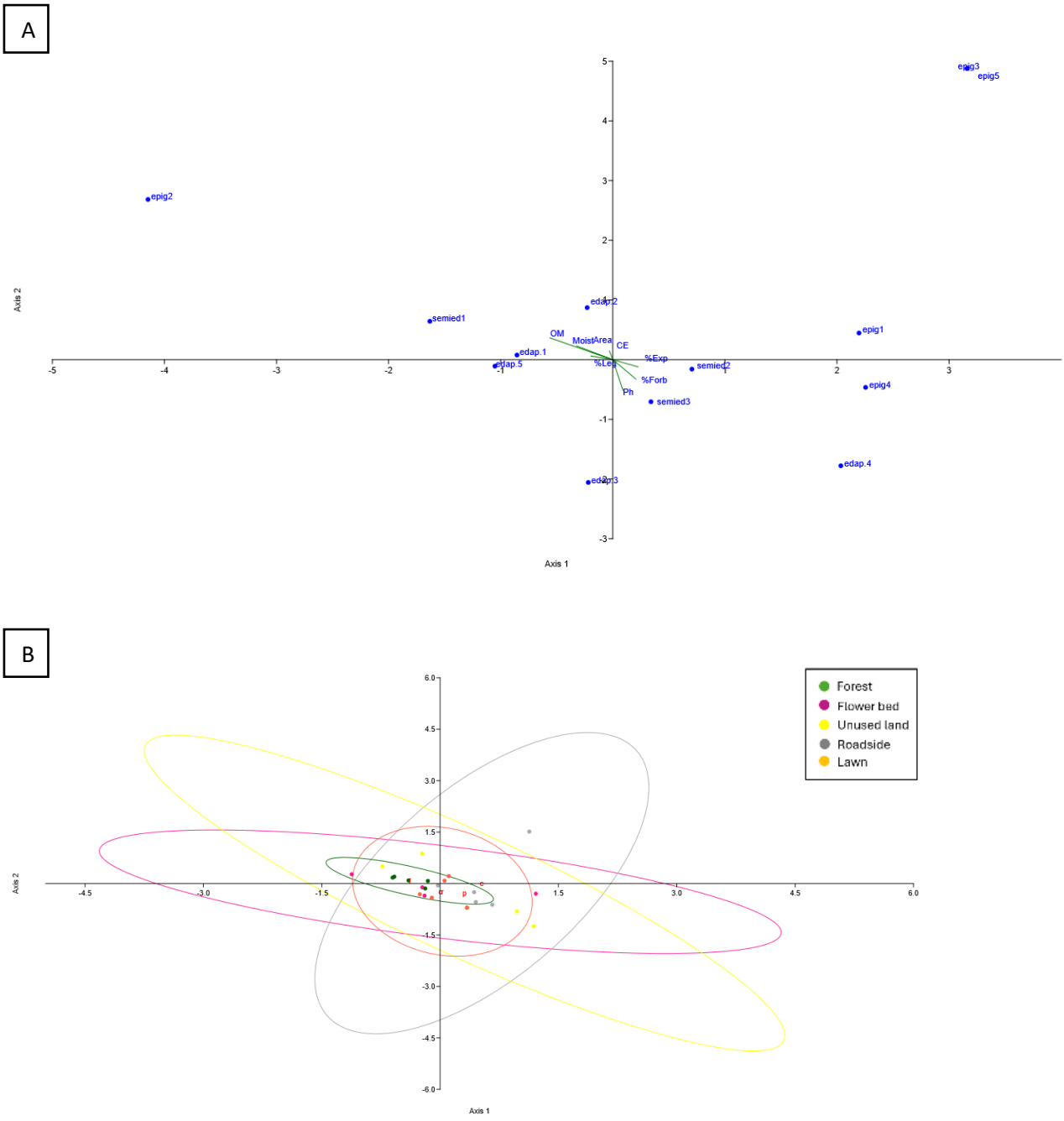


Annex 11. Canonical correspondence analysis of the influence of the environmental variables on Collembola parameters. Eigenvalues were 0.05402 and 0.007795 for the first and the second axis, respectively. A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and the mean trait value, percentage of edaphic collembolans and percentage of Acari/Collembola (Triplot amplitude: x1). (B) overlapping of 95% ellipses

for the land use groups. mT: mean trait value; %Col_edaf: percentage of edaphic collembolans; Acar/Colem: percentage of Acari/Collembola; OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.



Annex 12. Canonical correspondence analysis of the influence of the environmental variables on nematode taxonomical diversity. Eigenvalues were 0.12 and 0.09364 for the first and the second axis, respectively. Overlapping of 95% ellipses for the land use groups.



Annex 13. Canonical correspondence analysis of the influence of the environmental variables on collembolans morphotypic diversity. Eigenvalues were 0.2596 and 0.1782 for the first and the second axis, respectively. A) Canonical correspondence analysis bi-plot representing the environmental variable vectors and collembolans morphotypic diversity (Triplot amplitude: x1). (B) overlapping of 95% ellipses for the land use groups. OM: Organic Matter; pH: pH; CE: Electrical Conductivity; Moit: Moisture, %Leg: percentage of legume cover, %Forb: percentage of forb cover, %Exp: percentage of exposed soil; Area: Area.