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Ecological condition and functioning of urban soils in public green spaces: a multi-dimensional assessment in Milan, Italy

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CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

General introduction and thesis outline

Urban green spaces represent some of the few remaining patches of unsealed soil within densely built urban environments, where natural elements and ecological processes can still persist despite intense human pressure and are therefore essential for sustaining ecological functions in cities. In this context, soils in urban green spaces play a crucial role, as they may provide a wide range of ecosystem services (Morel et al., 2015). These include flood prevention through water infiltration, microclimate regulation and mitigation of the urban heat island effect, carbon storage, food provision through urban agriculture, support for biodiversity, and recreational benefits for city residents (O’Riordan et al., 2021). Despite this, the ecological value of urban soils is still poorly recognized by city managers and urban planners (Blanchart et al., 2018).

Urban soils have long been regarded as highly altered and characterised by low ecological quality overall (Morel et al., 2015; Pouyat et al., 2020). However, the growing body of scientific research on urban soils has progressively revealed a more complex reality. Within the same urban context, highly altered and degraded soils may coexist with soils that are less disturbed and retain relatively good ecological characteristics, in some cases comparable to those of semi-natural soils (Pouyat et al., 2007).

Urban soils are characterised by a high degree of heterogeneity, making it difficult to define the characteristics of a “typical” urban soil (Pouyat et al., 2007). Their current properties do not depend solely on present-day differences in land use, vegetation cover, and management practices, but also reflect land-use legacies and past processes and interventions linked to the site-specific history of each area (Pickett and Cadenasso, 2009; Ziter and Turner, 2018). As a result, the properties of urban soils are difficult to predict (Pouyat et al., 2007). In some cases, variations in soil properties can be observed along an urban–peri-urban gradient, reflecting differing levels of anthropogenic pressure (Foti et al., 2021). However, such patterns are not consistently expressed, highlighting the strong influence of site-specific legacies on local soil conditions.

Despite increasing scientific interest, several key dimensions of urban soil systems remain insufficiently explored. Substantial knowledge gaps persist regarding, for example, soil compaction (Binner et al., 2024) and soil biological communities, including soil fauna (Guilland et al., 2018). In addition, some aspects of urban soil functioning remain poorly documented, such as the capacity of soils in urban green spaces to infiltrate rainfall (Yang and Zhang, 2011; Wang et al., 2018).

Soil compaction is a widely reported form of physical degradation in urban environments, with broad ecological implications for soil functioning (Yang and Zhang, 2015). Increased bulk density reduces air permeability, limits water infiltration capacity and restricts root growth. Together, these changes alter

habitat suitability for soil fauna (Beylich et al., 2010). Urban soils have often been associated with high levels of compaction (Lehmann and Stahr, 2007); however, recent studies suggest that such generalised assumptions may not always be supported, highlighting the need for further investigations to assess the actual extent of soil compaction at the city scale (Edmondson et al., 2011; Paradelo et al., 2025). Moreover, compaction, linked for example to human trampling or mechanical actions, is a dynamic process to which virtually all urban soils may be exposed over time, which further underlines the importance of studying soil compaction in urban soil research (Burghardt et al., 2015; Pouyat et al., 2020).

Soil fauna represents a fundamental biological component of soils. Mirroring the strong heterogeneity observed in urban soils, edaphic fauna communities in cities may vary widely, with urban environments potentially supporting also locally rich and abundant assemblages (Schmidt, 2024). Among soil organisms, earthworms and soil microarthropods are particularly relevant, as they contribute to essential soil functions processes, including soil structure modification and nutrient cycling through detrital food webs (Lavelle et al., 2006; Blouin et al., 2013). Through their influence on soil structure, earthworms may also influence soil water infiltration processes (Edwards and Arancon, 2022). Despite their ecological importance, soil fauna in urban environments has received relatively limited attention, and current knowledge of how edaphic communities respond to urbanisation remains incomplete (Guilland et al., 2018).

An important aspect of urban soils concerns their capacity to support key ecosystem processes, such as water infiltration. In urban environments, soils in green spaces may contribute to the overall permeability of urban areas and thus have the potential to regulate surface runoff and flood risk at the city scale (Gill et al., 2007; Phillips et al., 2019). However, the capacity of urban soils to infiltrate rainfall remains insufficiently documented, particularly in relation to the physical and biological soil characteristics that may constrain or enhance this process (Wang et al., 2018).

Interpreting the environmental consequences of urbanisation for soil ecosystems requires the joint consideration of physical, chemical and biological soil dimensions within the same urban context, as well as their interactions in shaping soil functions. However, such integrated approaches remain relatively uncommon (e.g., Joimel et al., 2016, 2017; Horváth et al., 2021).

This thesis investigates urban soils within public green spaces of the city of Milan, Italy, by combining the assessment of soil physical and chemical properties, soil fauna, and water infiltration. Milan is the second largest Italian city and is located within one of the most densely urbanised regions of Europe. The city has a long history of human activity, which has profoundly shaped the territory. Despite this, published information on urban soils in Milan to date remains extremely limited. Data on soil fauna were lacking, while the few studies available on soil properties (Canedoli et al., 2020) and water infiltration (Galli et al.,

2021) addressed specific aspects or were limited to particular site contexts. This combination of intense urbanisation and limited available information makes Milan a suitable case study for exploring soil characteristics and functioning in a highly urbanised context, illustrative of a medium-sized European city.

The study was conducted in publicly accessible green areas across the city. A set of green area categories was defined to capture differences in vegetation type, management intensity, land-use history, levels of human use, and position within the urban context most frequently observed across Milan. These categories guided the experimental design throughout the entire thesis.

The thesis was conceived to provide an overall assessment of urban soil conditions in the city of Milan, through the joint analysis of multiple soil dimensions. To delimit the scope of investigation, soil compaction and its potential implications for soil ecological functioning were initially identified as the central research question, given its potential relevance in urban environments. This initial focus guided the selection of the soil properties, biological components and soil processes included in the study. However, the subsequent assessment of soil properties showed that soil compaction was not a major limiting factor in the investigated urban green spaces. The research objectives were thus reframed towards a broader evaluation of the ecological condition and functioning of Milan's urban soils, while maintaining the same analytical approach and set of investigated components. In doing so, the thesis aims to improve understanding of how urban pressures affect multiple soil dimensions and to assess whether evidence supports assumptions of widespread ecological degradation of urban soils.

Specifically, the thesis aimed to:

- characterise the physical and chemical properties of urban soils, with particular attention to soil compaction;
- examine soil fauna biodiversity in urban green spaces, and identify soil and environmental drivers shaping community patterns in urban areas;
- assess biological soil quality using a soil fauna-based biological index and evaluate its ability to discriminate the effects of urban pressures on soils;
- investigate a key soil process in urban environments, namely water infiltration, and its relationship with physical and biological soil components.

These objectives were addressed in Chapters 2–5.

Chapter 2 provides an extensive assessment of the chemical and physical soil properties of Milan's public green spaces. A total of 60 sites was sampled throughout the city, spanning the different green area categories. Soils were investigated down to 40 cm depth, in order to capture also subsurface conditions. Measured properties included bulk density, penetration resistance, soil texture, pH, organic carbon, total nitrogen, and available phosphorus. The aim of this chapter is to investigate whether and how urban

pressure has altered soil characteristics, with a particular focus on soil compaction, and to discuss the potential implications of these alterations for soil ecological functioning.

By providing an overview of the physical and chemical conditions of soils in Milan's public green spaces, this chapter establishes the background knowledge on which the subsequent studies are built. This characterisation in fact was used to select a subset of 15 sites for the investigation of soil fauna and water infiltration.

Chapters 3 and **4** add a biological perspective to the assessment of urban soils. **Chapter 3** investigates earthworm communities, which were characterised in terms of abundance, biomass, and functional and taxonomic composition. In addition, the factors shaping community patterns were examined by considering soil properties (including heavy metal concentrations), vegetation cover, and landscape configuration, in order to assess the effects of urbanisation on earthworms. To our knowledge, this chapter provides the first systematic dataset on earthworm communities in urban environments in Italy.

Chapter 4 focuses on soil microarthropods. The QBS-ar index (Soil Biological Quality index based on microarthropods; Parisi et al., 2001, 2005) was used to characterise microarthropod communities and to assess soil biological quality across Milan's public green spaces. Variations in QBS-ar values among green area categories were examined in relation to soil properties, management intensity and site context, to evaluate how effectively the index captures the effects of urban pressures on soils. This chapter also discusses the usefulness and limitations of QBS-ar as a tool for assessing biological soil quality in heterogeneous urban environments.

Chapter 5 examines water infiltration as a key soil process in urban ecosystems. Water infiltration rates were quantified and compared across green area categories. The results were discussed in relation to typical rainfall conditions experienced in Milan, to assess the potential of soils in urban green spaces to infiltrate precipitation. In addition, the chapter investigates the physical and biological factors potentially controlling water infiltration in urban soils, with particular attention to the role of soil compaction and earthworms.

Finally, **Chapter 6** provides some general conclusions on the results obtained and discusses future perspectives.

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CHAPTER 2

SOIL CHEMICAL AND PHYSICAL PROPERTIES

The results presented in this chapter have been published in:

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Abstract

Urban soils play a crucial role in supporting ecosystem services, yet they are often overlooked or perceived as degraded. This study provides an assessment of physical and chemical properties of soils in public green spaces in Milan, Italy—a representative medium-sized European city—with particular attention to soil compaction. Sixty georeferenced plots were sampled across five categories of publicly accessible green areas differing in vegetation cover, management intensity, and human pressure. Measurements included bulk density (BD), penetration resistance (PR), pH, organic carbon (SOC), total nitrogen, available phosphorus (AvP), and texture.

Milan's soils generally retained favourable ecological properties. BD was low (mean \pm sd: 1.03 ± 0.13 g cm⁻³), below thresholds limiting root growth and soil biota, and PR profiles showed no critical compaction. However, BD and PR varied across categories: values were lowest in peri-urban forests and highest in central parks and urban green islands, reflecting vegetation, management, and recreational pressure differences. SOC was relatively high in the topsoil (mean \pm sd: $3.23 \pm 0.75\%$), supporting fertility and carbon storage, with no significant differences among categories. Conversely, pH and phosphorus varied: peri-urban sites had acidic to sub-acidic conditions and moderate AvP levels, while central parks and urban islands showed near-neutral to slightly alkaline pH and elevated AvP, indicating stronger anthropogenic influence.

These findings challenge the widespread assumption that urban soils are uniformly compacted and degraded. Soils in Milan's green spaces preserve properties that sustain ecosystem services, with differences reflecting management intensity, recreational use, and land-use history. The framework adopted here can be applied in other cities to guide soil protection and support sustainable urban planning.

2.1. Introduction

Urban areas currently host 55% of the global population, and this proportion is expected to increase (United Nations, 2019). Urban expansion not only increases impervious surfaces but also contributes to land fragmentation through urban sprawl (Burghardt et al., 2015). The scattered unsealed areas that persist within urbanized territories remain the only ones capable of providing essential ecosystem services (ES) for the well-being of city inhabitants. In this context, urban soils play a crucial role in improving the environmental quality of cities. Despite anthropogenic disturbance, urban soils—like natural and semi-natural ones—provide numerous ecosystem services (Lehmann and Stahr, 2007; Morel et al., 2015; Pouyat et al., 2020). These include flood prevention through water infiltration, microclimate regulation, heat island effect mitigation, carbon storage, food provision via urban agriculture, support for biodiversity, and recreational benefits for city residents (O’Riordan et al., 2021).

Nevertheless, soils are often neglected in urban ecosystem management, being seen as a resource for intensive use rather than a natural heritage that must be preserved to maintain its ecological functionality (Morel et al., 2015; Pouyat et al. 2020). There is a persistent prejudice that all urban soils are highly altered and of poor quality (Morel et al., 2015; Pouyat et al., 2020). However, the situation is more complex than commonly assumed. Although all urban soils are affected by human activities, the extent of alteration and degradation can vary considerably within the same urban context, where highly altered soils can coexist with pseudo-natural soils (Pouyat et al., 2007; Chaurasia et al., 2024). Some soils are deeply transformed, with chemical and physical properties that differ drastically from non-urban soils (e.g., high compaction, alkaline pH, and the presence of artefacts and residues from human activities) and hinder biological activity and plant growth. Contamination can also be a concern in some cases (Norra and Stüben, 2003; Horváth et al., 2015). In contrast, some urban soils remain relatively undisturbed and exhibit properties more similar to those of natural soils, such as low bulk density, high organic matter content, and rich biodiversity (Pouyat et al., 2020).

Urban soils are characterized by high spatial heterogeneity, even over short distances (i.e., within just a few tens of meters, Greinert, 2015), making it difficult to define the characteristics of a “typical” urban soil (Pouyat et al., 2007). This heterogeneity is driven by current differences in land use, vegetation cover, and management practices, as well as land use legacies and historical processes that have shaped the different patches of soil throughout the city’s history (Pickett and Cadenasso, 2009; Ziter and Turner, 2018; Paradelo et al., 2021; Chaurasia et al., 2024). Among these, the time since the actual land use has been established is a key factor that can sometimes be crucial in explaining differences in soil properties (Scharenbroch et al., 2005; Greinert, 2015). In the heterogeneous mosaic of soil patches that compose urban landscapes, a

general gradient of soil property alteration can sometimes be observed, following the urbanization gradient from rural to urban areas (Foti et al., 2021; Whitehead et al., 2021).

Recently, urban soils have received increasing attention both within the scientific community and in European policy frameworks such as the EU Soil Strategy for 2030 (European Commission, 2021). However, significant knowledge gaps remain, and current research does not yet provide a comprehensive overview of their status. While topics such as heavy metal contamination and soil organic carbon have been more extensively studied, others, such as soil compaction and biodiversity, remain underexplored (Binner et al., 2024).

Compaction can significantly contribute to soil degradation in urban environments (Lehmann and Stahr, 2007; Morel et al., 2015), leading to alterations in soil structure that result in increased bulk density and reduced porosity. These changes limit air circulation and promote anaerobic conditions and enhance denitrification (Li et al., 2014). Reduced infiltration capacity contributes to increased surface runoff and a higher risk of flooding (Gregory et al., 2006; Johnston et al., 2016). Compaction also creates unsuitable habitat conditions for soil microorganisms and fauna, compromising soil biological activity (Beylich et al., 2010; Devigne et al., 2016). Moreover, increased soil resistance to penetration hinders root development and thus affect plant growth (Yang and Zhang, 2015). Soil compaction in urban environments results from various factors, including high foot traffic in green spaces, vehicles used for greenery maintenance, heavy machinery used for construction and landscaping, and intentional compaction for infrastructure development (Yang and Zhang, 2015).

Urban soils have often been associated with high levels of compaction (Lehmann and Stahr, 2007), but more recent studies suggest that this assumption is not universally valid. One reason for this perception is that many studies reporting high bulk density (BD) values have focused on specific, highly disturbed areas rather than providing a comprehensive view of urban soils (Edmondson et al., 2011). Large-scale investigations on urban areas, on the other hand, reveal a more complex and variable picture, showing that soil compaction is not always widespread and largely depends on land use, vegetation cover, and the intensity of human activity. Several studies have reported that compaction tends to be confined to zones of high-intensity use, while bulk density values across the entire urban area generally remain low (Foti et al., 2021; Paradelo et al., 2025), sometimes even falling below those observed in the surrounding agricultural areas (Edmondson et al., 2011). Conversely, other authors (Scharenbroch et al., 2005; Chaurasia et al., 2024) have documented widespread soil compaction within the city, with bulk density values often reaching levels known to restrict root growth (USDA, 2023).

This study addresses the current knowledge gap on urban soils by providing an extensive assessment of their physical and chemical properties in public green spaces, with a particular focus on soil compaction,

using the city of Milan as a representative case study of a medium-sized European city. We selected a set of green space categories to reflect the most characteristic situations across the city, trying to capture differences in vegetation type, management intensity, land-use history, levels of human use, and position within the urban context. The aim was to investigate whether urban pressure has altered soil characteristics, and whether such alterations, if present, may influence its ability to support ecological functions.

2.2. Materials and methods

2.2.1 Study area

The investigation was carried out in the city of Milan, located in northern Italy, in the central-western part of the Po Valley's alluvial plain. The landscape is predominantly flat, with an average elevation of about 100 m. The climate is classified as continental, with a mean annual temperature of 13.0 °C and a mean annual precipitation of 920 mm. Monthly mean temperatures range from 2.3 °C in January to 23.8 °C in July (1991–2021). The predominant soil types in the lowland area where Milan is located are Luvisols (primarily Dystric and Gleyic) and Cambisols (mostly Skeletic and Gleyic) (ERSAL 1993, 1999).

Milan's urban structure reflects its long history of development, which has profoundly shaped the territory. The surrounding area has historically been an important agricultural region. The city evolved from a medieval core built over the former Roman settlement, with successive expansions radiating outward from the center. The districts surrounding the old core were largely developed in the late 19th and early 20th centuries, when Milan had become a major industrial center, a role it maintained until the onset of deindustrialization in the 1970s. The outer belt expanded mainly after World War II through rapid urban sprawl, converting former rural and agricultural areas into densely built environments (Canedoli et al., 2017). As a result, the present-day city shows a clear gradient from the dense historical center to more heterogeneous peripheral areas, where residential, industrial, and semi-natural spaces coexist.

Today, Milan and its metropolitan area are among the largest and most densely populated urban regions in Italy. The city hosts 1,407,044 residents, resulting in a population density of 7,741 people per square kilometer. Public green spaces cover approximately 25 square kilometers, accounting for nearly 14% of the city's total area (Municipality of Milan, 2024).

2.2.2 Selection of green area categories

Five categories of public green areas were defined, based on the vegetation cover, the type of green space, and the levels of public use most frequently observed across the city. In the absence of objective data, the intensity of green space use was evaluated qualitatively based on factors that can be indicative of varying levels of anthropogenic pressure across the city, including green area size, location (historic centre vs. peri-urban areas), and history of land use.

The selected categories include grasslands in urban parks inside and outside the historic centre (UPC and UP respectively); grasslands in urban green islands (UGI); grasslands in peri-urban parks (PP), and forests in peri-urban parks (PPf). Central parks (UPC) are historic green areas that are intensively managed and subject to long-term recreational use, with high foot traffic. Urban parks outside the centre (UP) are more recent and are generally less frequented than historic parks, but are managed similarly. Urban green islands (UGI) are small, publicly accessible vegetated spaces —such as green spaces within urban squares and traffic islands— designed for both ornamental and recreational purposes. Due to their small size and proximity with roads and buildings, they are often exposed to high localized disturbance. Peri-urban parks (PP and PPf) are extensive areas with lower management intensity and reduced human pressure and often retain semi-natural features.

All green spaces considered in this study are publicly accessible and not subject to trampling restrictions. However, patterns of use differ: in forested sites, visitors generally remain on designated paths, whereas in grasslands, people tend to use the entire surface.

2.2.3 Soil sampling and compaction measurements

A total of 60 sampling locations were selected and distributed across the five green area categories throughout the city (Fig. 1). The number of locations per category was as follows: PPf = 9, PP = 21, UGI = 10, UP = 10, and UPC = 10. At each location, a georeferenced experimental plot (4 x 4 m) was defined for soil sampling and soil compaction measurements. The plots were located on surfaces representative of the prevailing conditions within each green space, while avoiding localized features (e.g., footpaths, edges, or temporary alterations) that may not reflect the general characteristics of the area.

Soil samples were collected at three depths: 0–10 cm, 10–20 cm, and 20–40 cm, using a 4-cm-diameter auger. For each depth, a composite sample was obtained by combining five subsamples, following the standard LUCAS (Land Use and Cover Area frame Survey) protocol: one central subsample (at the center of the plot) and four subsamples placed along the axes, each 2 m from the centre.

Soil compaction status was assessed by measuring soil bulk density and soil resistance to penetration. Bulk density (BD) was measured in the top 5 cm by taking three subsamples of undisturbed soil with a cylindrical sampler (volume = 100 cm³, total composite sample volume = 300 cm³). Soil resistance to penetration (PR) was measured using a field penetrometer (*FieldScout SC 900 Soil Compaction Meter, Spectrum Technologies, Inc., Plainfield, IL*), which records soil resistance at increasing depths (with 2.5 cm resolution), up to a maximum depth of 45 cm. Five repeated penetrometer measurements were taken within each experimental plot and averaged to obtain a single value. To account for the influence of soil moisture on PR, the soil water content was measured at 0–10 cm depth using a TDR sensor (*FieldScout TDR 150 Soil Moisture Meter, Spectrum Technologies, Inc., Plainfield, IL*).

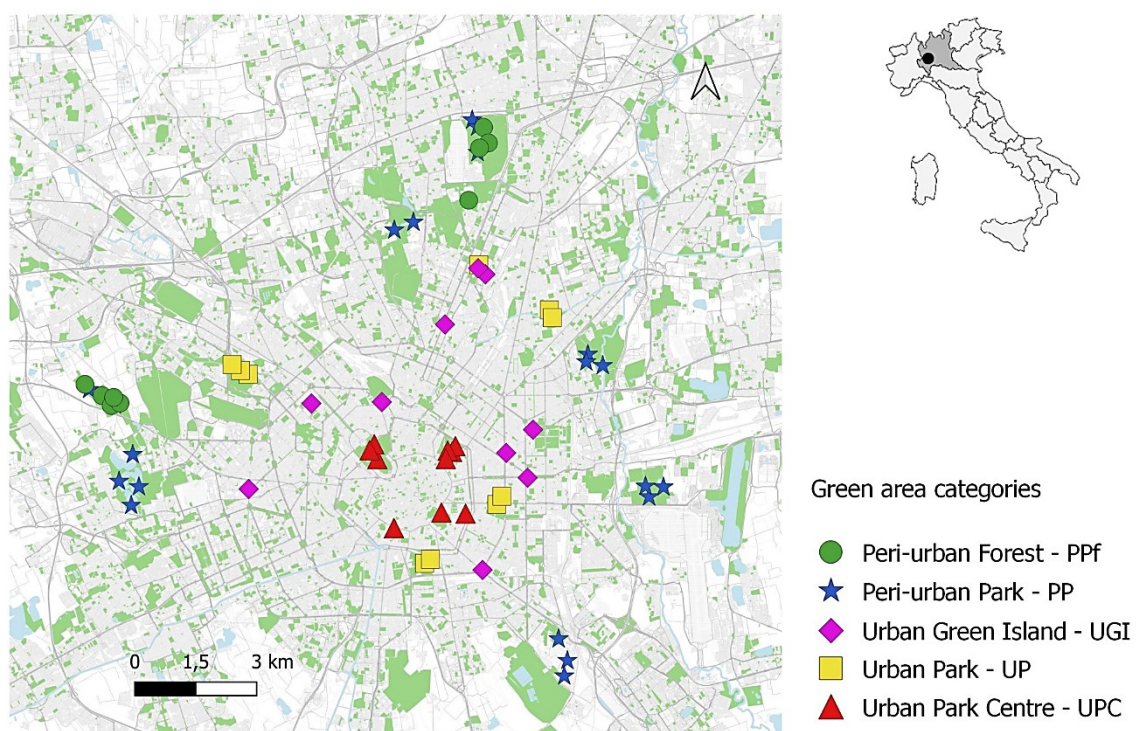


Figure 1. Location of the 60 sampling plots across Milan. Sites are grouped by green area category, as indicated in the legend.

2.2.4 Laboratory analyses

A total of 178 soil samples were air-dried, sieved (2 mm mesh) and analysed to determine soil organic carbon (SOC, after carbonate removal) and total nitrogen content (*Flash EA 1112 NCSoil, Thermo Fisher Scientific elemental analyzer, Pittsburgh, PA, USA*), pH in water (soil to water ratio of 1:2.5), particle-size distribution by sieving and sedimentation (Burt, 2004) (coarse sand: 0.1–2 mm; fine sand: 0.05–0.1 mm; silt: 0.002–0.05; clay, < 0.002 mm) and available phosphorus (Olsen et al., 1954; determined in the 0–10 cm layer only). Bulk density of fine earth was calculated on the 60 composite samples, correcting for the volume of rock fragments by separating the coarse fraction, weighing it, and measuring its volume.

2.2.5 Data processing and statistical analyses

To allow for direct comparison of soil PR measurements collected under varying soil moisture conditions, the data were corrected for differences in water content, since this parameter significantly impacts the values obtained (Busscher et al., 1997). The correction function was derived from a modified version of the procedure described by Duarte et al. (2022), using data from a non-compacted reference site, whose soil texture matched the predominant texture found in the sampling plots. At the reference site, both PR and water content were measured across a range of soil moisture conditions. This approach enabled us to isolate the effect of soil moisture on PR, independently of compaction. The correction function was then applied to all field data, adjusting PR values to a common reference water content corresponding to field capacity (FC = 24%).

Descriptive statistics (minimum, maximum, mean and standard deviation) were calculated for all measured soil properties in all the 60 plots. To ensure uniformity, PR values were averaged within the same three depth intervals as the other soil properties.

To test for differences among categories, linear mixed-effects models (LMMs) were fitted for each soil property (pH, SOC, N, P, BD, and PR in three layers), with green area category as the only fixed effect and location (i.e., a specific park) as a random effect (Schabenberger and Gotway, 2005). To further isolate the effect of green space category on soil compaction, a second set of LMMs was fitted for bulk density and penetration resistance. Unlike the previous models, these included not only green area category but also relevant soil parameters as fixed effects, with location included as a random effect. By accounting for key soil covariates, these models aimed to separate the anthropogenic effect from the influence of intrinsic soil characteristics. Covariates were selected using a stepwise model selection procedure based on Akaike's Information Criterion (AIC). Model assumptions were checked by visual inspection of diagnostic plots and by applying the Shapiro–Wilk test for normality. When needed, variables that were not normally distributed were log-transformed prior to analysis. Residual spatial autocorrelation was tested using Moran's I across all fitted models. All confidence intervals were calculated at the 95% level. When significant effects were found, pairwise comparisons were performed between the different green area categories using Tukey's Honestly Significant Difference (HSD) test.

All statistical analyses were performed in R (version 4.5.0, R Core Team 2025) using the *stats*, *nlme*, *spdep*, and *emmeans* packages.

2.3. Results

The main physical and chemical properties of soils from all 60 sampling plots are summarized in Table 1. Soil organic carbon content decreased markedly with increasing depth. In the topsoil (0–10 cm), SOC values were generally high (mean \pm SD: $3.23 \pm 0.75\%$), with values ranging from 2.01 to 4.98%. In deeper layers, SOC generally remained at moderate levels, although some plots exhibited very low values ($< 1\%$). Total nitrogen content followed a similar trend. The C:N ratio remained consistent across all green area categories, including both grasslands and forests. Average values were 11 in the surface layer and 10 at greater depths, with low variability between plots. However, some outliers were observed, including notably high values in one UGI plot. Regarding pH, mean values increased slightly with depth, from 6.2 ± 0.8 in the topsoil to 6.5 ± 1.2 in the deepest layer, consistently falling within the range typical of neutral to slightly acidic soils. However, across layers, there were also extremes that vary from very acidic (minimum = 4.5) to subalkaline conditions (maximum = 8.0). Bulk density averaged $1.03 \pm 0.13 \text{ g cm}^{-3}$, with maximum values up to 1.27 g cm^{-3} . Soil penetration resistance increased with depth. Average values were relatively moderate, but PR ranged widely across plots and layers, from 0.47 to 3.85 MPa. Available phosphorus in the topsoil (0–10 cm) was highly variable, ranging from 9 to 127 mg kg^{-1} across plots.

Table 1. Descriptive statistics of soil data (60 plots). BD = bulk density, PR = penetration resistance, SOC = soil organic carbon, tN = total nitrogen, AvP = available phosphorous. Layer: depth in cm.

| | Layer | Mean | SD | Min | Max |
|-----------------------------|-------|------|------|------|------|
| BD (g cm^{-3}) | 0–5 | 1.03 | 0.13 | 0.73 | 1.27 |
| PR (MPa) | 0–10 | 1.63 | 0.56 | 0.47 | 2.89 |
| | 10–20 | 2.31 | 0.66 | 0.69 | 3.85 |
| | 20–40 | 2.40 | 0.64 | 0.82 | 3.82 |
| pH | 0–10 | 6.2 | 0.8 | 4.9 | 7.7 |
| | 10–20 | 6.3 | 1.0 | 4.5 | 7.9 |
| | 20–40 | 6.5 | 1.2 | 4.5 | 8.0 |
| SOC (%) | 0–10 | 3.23 | 0.75 | 2.01 | 4.98 |
| | 10–20 | 1.75 | 0.61 | 0.63 | 3.20 |
| | 20–40 | 1.20 | 0.42 | 0.30 | 2.20 |
| tN (%) | 0–10 | 0.30 | 0.07 | 0.11 | 0.46 |
| | 10–20 | 0.17 | 0.05 | 0.04 | 0.28 |
| | 20–40 | 0.12 | 0.05 | 0.04 | 0.28 |
| C:N | 0–10 | 11 | 1 | 9 | 18 |
| | 10–20 | 10 | 2 | 7 | 17 |
| | 20–40 | 10 | 2 | 5 | 18 |
| AvP (mg kg^{-1}) | 0–10 | 33 | 25 | 9 | 127 |

Soil texture classes in the topsoil (0–10 cm) are shown in the textural triangle (Fig. 2). In this layer, all plots belonging to UP, UPC, and PPf exhibited exclusively loam, sandy loam, or silty loam textures. In contrast, PP and UGI displayed somewhat greater variability. With increasing depth, soil texture generally remained similar to that of the top layer, except for UGI plots, where the higher variability observed in the surface horizons tended to decrease in the deeper layers.

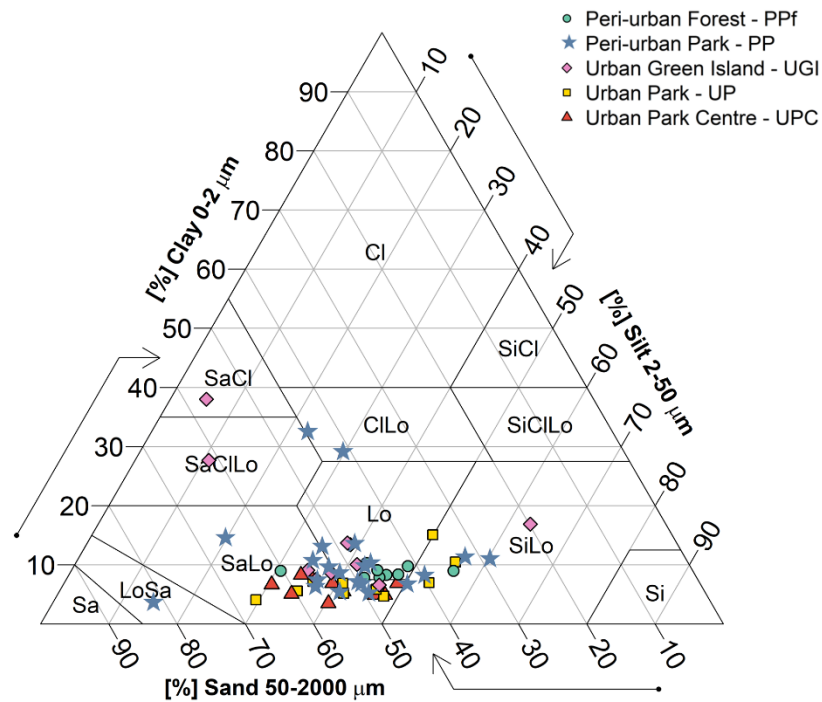


Figure 2. Soil texture classes (USDA) of soil samples collected in the first layer (0-10 cm).

Fig. 3 displays the mean soil penetration resistance profiles by green area category, down to a depth of 45 cm. In all grassland categories (PP, UGI, UP and UPC), PR increased sharply from the soil surface to a depth of approximately of 10–12.5 cm, after which it remained relatively stable until the deeper layers, where more pronounced variations were observed. These categories exhibited highly similar profiles throughout most of the soil depth, with mean PR values remaining always below 2.75 MPa. In contrast, the profile for PPf showed a more gradual and nearly linear increase in PR with depth, without the initial rapid rise observed in the grassland categories. PR values in PPf were consistently much lower, ranging from about 0.50 MPa at the surface to 1.75 MPa at 40 cm, clearly distinguishing this category from the others.

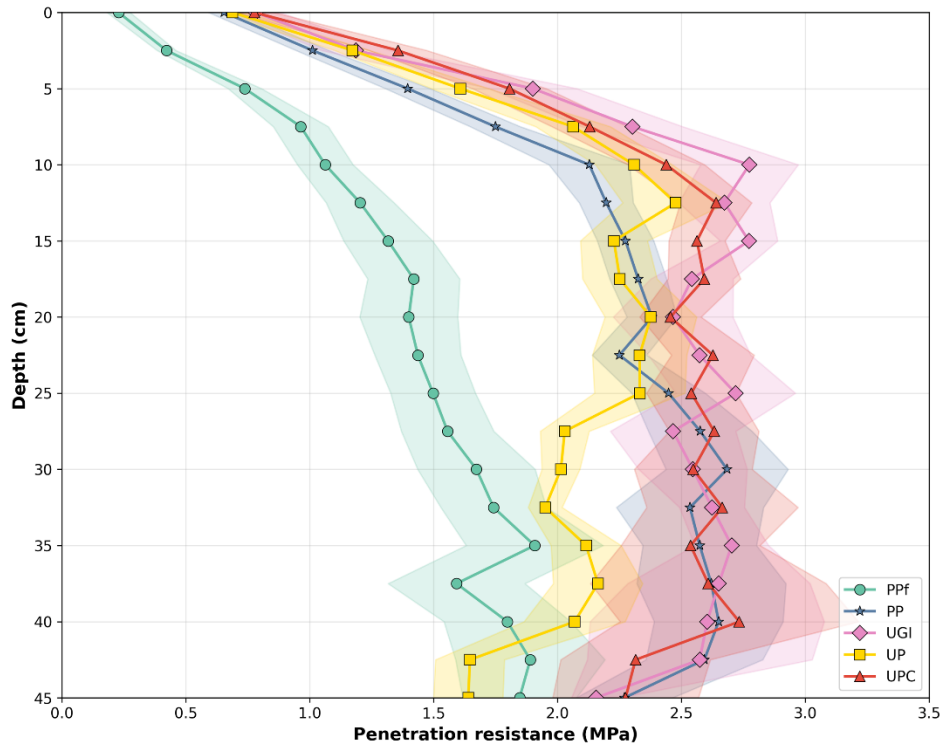


Figure 3. Soil penetration resistance profiles by green area category. Mean values (solid lines) and standard error (shaded bands) are reported.

Complete outputs of the LMMs are reported in Supplementary Materials. Here we summarize the main results. Linear mixed models revealed significant differences in pH between green area categories in all three investigated soil layers (Fig. 4). In the upper two layers, a very similar pattern emerged: peri-urban plots (PP and PPF) exhibited the lowest mean pH values (0–10 cm: mean = 5.9, 95% CI [5.6–6.3] for PP; mean = 5.7, 95% CI [5.2–6.2] for PPF), which differed significantly from those of UPC and UGI, both characterized by the highest values (0–10 cm: mean = 6.9, 95% CI [6.4–7.5] for UPC; mean = 6.7, 95% CI [6.3–7.2] for UGI). UP plots had intermediate pH values (0–10 cm: mean = 6.4, 95% CI [5.9–6.9]). In the deepest layer (20–40 cm), the differences between the categories were even more pronounced. PPF exhibited significantly lower pH values than all the other categories (mean = 5.4, 95% CI [4.9–6.0]), including PP. In contrast, UPC had the highest pH values, reaching the sub-alkaline range (mean = 7.6, 95% CI [6.8–8.4]), and differed significantly from both peri-urban park categories.

A similar pattern of differences was observed for available phosphorus as for pH. The LMM showed significant differences among green area categories (Fig. 4). PP and PPF exhibited the minimum values (mean = 20, 95% CI [16–26] mg kg⁻¹ and mean = 18, 95% CI [12–26] mg kg⁻¹, respectively), which were significantly lower than those observed in UGI and UPC, which showed the highest values (mean = 47, 95% CI [32–68] mg kg⁻¹ and mean = 43, 95% CI [30–62] mg kg⁻¹, respectively). No significant differences were found for UP.

The linear mixed-effects models detected no significant differences among green area categories for soil organic carbon, total nitrogen and C:N ratio; descriptive statistics for these variables (means and standard deviations by category) are reported in Table S1 (Supplementary Materials).

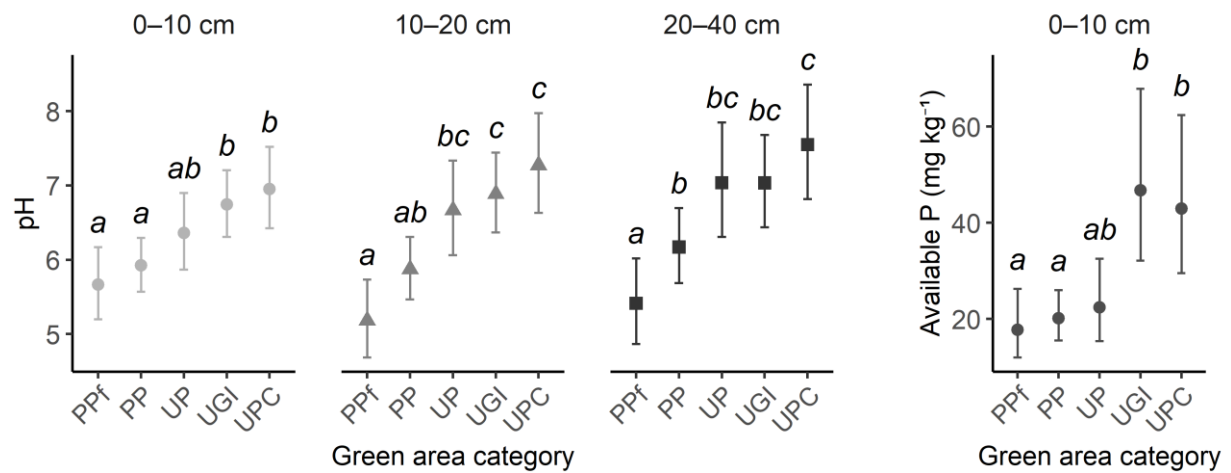


Figure 4. Estimated marginal means ($\pm 95\%$ CI) for pH and available P from LMMs including green area category as fixed effect. Letters in italic indicate significant pairwise differences among green area categories (Tukey post hoc test).

The best-fitting LMM for BD included green area category and soil organic carbon in the top layer (0–10 cm) as fixed effects (Fig. 5). SOC had a significant negative effect on BD (estimate = -0.039 , $p = 0.044$). BD was significantly lower in forests (mean = 0.90 , 95% CI [0.83 – 0.98] g cm^{-3}) compared to all grassland categories. PP (mean = 0.98 , 95% CI [0.93 – 1.03] g cm^{-3}) had lower values than UPC (mean = 1.15 , 95% CI [1.08 – 1.22] g cm^{-3}) but did not differ significantly from UP (mean = 1.08 , 95% CI [1.01 – 1.15] g cm^{-3}) or UGI (mean = 1.08 , 95% CI [1.02 – 1.15] g cm^{-3}). UP and UGI also did not differ significantly from either PP or UPC.

For PR in the topsoil (0–10 cm), the best-fitting model included green area category as the only fixed effect. In the 10–20 cm layer, soil organic carbon, sand, and clay were selected in addition to the green area category. For the deepest layer (20–40 cm), sand, clay and green area category were included.

In the second and third soil layers, sand content was positively associated with PR (PR 10–20 cm: estimate = 0.002 , $p = 0.019$; PR 20–40 cm: estimate = 0.003 , $p = 0.002$). In all three layers, green area category had a significant effect on PR. In the topsoil (0–10 cm), a clear pattern emerged among green area categories (Fig. 5): PR was lowest in PPf (mean = 0.80 , 95% CI [0.52 – 1.08] MPa), which was significantly lower than all other categories. PP (mean = 1.57 , 95% CI [1.39 – 1.75] MPa) showed lower PR than UGI (mean = 2.04 , 95% CI [1.78 – 2.31] MPa) but did not differ significantly from UP (mean = 1.79 , 95% CI [1.53 – 2.05] MPa) or UPC (mean = 1.93 , 95% CI [1.67 – 2.20] MPa). UP and UPC had intermediate values, not significantly different from either PP or UGI. The highest PR values were observed in UGI. In the second (10–20 cm)

and third (20–40 cm) layers, the only significant difference that remained was between peri-urban forests and all other categories, with PR in PPf (mean = 1.32, 95% CI [1.01–1.63] MPa at 10–20 cm; mean = 1.68, 95% CI [1.35–2.01] MPa at 20–40 cm) being significantly lower than in any other group (Fig. 5).

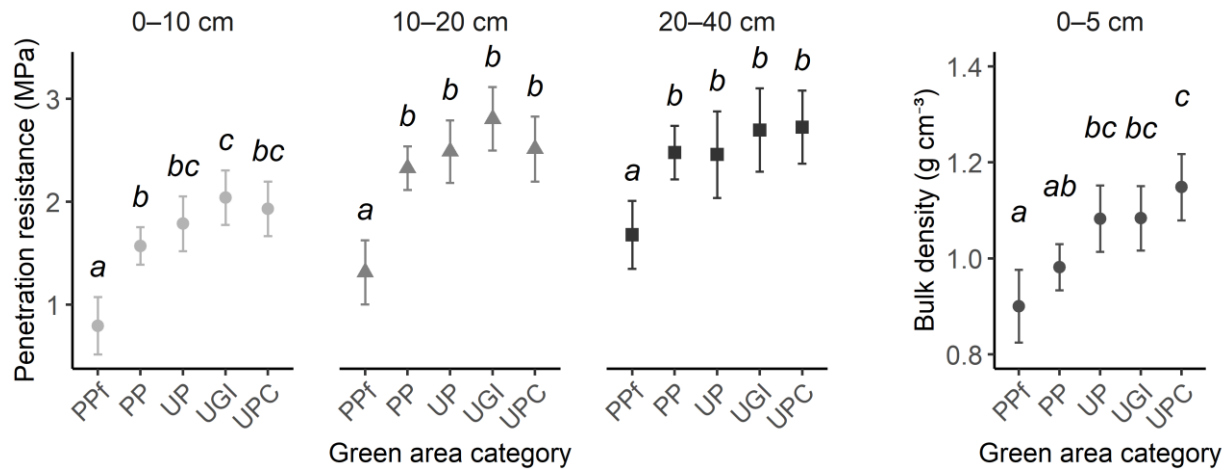


Figure 5. Estimated marginal means ($\pm 95\%$ CI) for PR and BD from LMMs including green area category and selected soil covariates as fixed effects. Letters in italic indicate significant pairwise differences among green area categories (Tukey post hoc test).

2.4. Discussion

This study represents one of the first extensive investigations of the chemical and physical properties of soils in the city of Milan, aside from the work of Canedoli et al. (2020), which focused specifically on SOC stocks. Our aim was to provide an overview of the soils in Milan's public green spaces, by considering the combined influence of vegetation type, management practices, intensity of human use, land-use history, and location within the urban context. More broadly, the goal was to assess the quality of urban soils in Milan, and to evaluate whether their properties are consistent with the ecological functioning and the delivery of ecosystem services.

Our study focuses on a well-defined subset of urban soils: publicly accessible green spaces designed for recreational and ornamental purposes, including parks, neighborhood greens, and small vegetated areas such as traffic islands. Excluding heavily impaired or marginal urban land types has a direct influence on the results. Many urban soil studies which reported altered soil properties included highly disturbed sites such as barren land, construction areas, or roadside verges (Gregory et al., 2006; Park et al., 2010; Zandybay et al., 2024). Consequently, comparing findings across studies requires careful attention to the characteristics of the sampled sites and the context in which the research was conducted.

Soil texture across Milan's public green spaces appeared relatively uniform, with most samples falling into loam, sandy loam, or silty loam classes that are typical of the region's natural soils, suggesting limited soil disturbance. An exception was observed in UGI, where surface horizons showed greater heterogeneity in texture. Due to their ornamental nature and small size, these spaces are often subject to frequent redesigns, maintenance works, and surface transformations. In most cases, textural variability decreased with depth, indicating that disturbances are primarily confined to the topsoil. As is commonly reported in urban soil studies (Morel et al., 2015), anthropogenic activities tend to affect only the upper horizons, while deeper layers generally retain their original texture.

Brick fragments were commonly observed in small quantities across sites. Other anthropogenic materials (such as glass, plastic, concrete, or metal) were sporadic and localized. Overall, the total amount of artefacts was low, suggesting limited signs of artificialization despite the urban context. According to WRB classification criteria, no Technosols were identified. Our sampling depth (0-40 cm) prevented complete WRB classification; deeper sampling would likely reveal Cambisols and Luvisols typical of the Milan area. The sampled surface horizons could be described as those of "pseudo-natural soils" (Morel et al., 2015).

As expected, SOC content decreased with depth. Surface soils (0–10 cm) showed relatively high concentrations. Low values were uncommon and were mostly found in deeper horizons. These findings are consistent with those of previous studies (Edmondson et al., 2012; Foti et al., 2021) and confirm that soils in Milan's green spaces have good levels of organic matter, supporting soil fertility and structural stability. In addition, they contribute significantly to carbon storage, as highlighted by Canedoli et al. (2020). Moreover, SOC concentrations are similar to those of permanent grasslands and exceeded those of agricultural soils in the areas surrounding the city—an outcome frequently highlighted in the literature (Vasenev et al., 2013; Paradelo et al., 2021).

No significant differences in SOC were detected across green area categories, consistent with findings from other cities (Pouyat et al., 2007; Paradelo et al., 2021). Even in more central and potentially disturbed contexts, such as UPC and UGI, SOC levels remain comparable to those in peri-urban categories. Interestingly, SOC content in forested areas was not higher than in grass-covered ones, probably due to a legacy effect. Although these forest patches were established nearly 50 years ago, SOC content still bears the imprint of the former agricultural land use. This supports the idea that SOC responds slowly to changes in vegetation cover, with soil transformations occurring over longer timescales than vegetation shifts (Poeplau et al., 2011). This is further supported by the C:N ratio, which showed similar values in both forested and grass-covered sites, suggesting that not only the quantity, but also the quality of the soil organic matter remains similar across these green areas.

As expected, pH values increased with depth, indicating relatively undisturbed soil profiles. Lower pH values—acidic to sub-acidic—were found in peri-urban grasslands and especially in forested areas, consistent with their vegetation cover and limited anthropogenic disturbance. In contrast, higher values—near neutral or slightly alkaline—were observed in the more intensively managed and centrally located sites (UGI and UPC), likely reflecting the influence of calcareous fill materials, construction residues, or urban fertilization practices (Yang and Zhang, 2015). Intermediate values were recorded in UP sites, possibly due to greater internal variability across parks. Differences became more pronounced in deeper horizons, with PPF and UPC representing the two extremes. Similar trends have been reported in previous urban soil studies (Greinert et al., 2015) which, as in our case, observed pH values within ecologically optimal ranges. This challenges the generalized assumption that urban soils are typically excessively alkaline (Yang and Zhang, 2015).

Available phosphorus concentrations showed patterns consistent with those observed for pH, with higher values recorded in more intensively managed and centrally located green spaces (UGI and UPC). These areas are subject to frequent – historical or ongoing – fertilization practices, which likely contribute to the elevated P levels. In contrast, lower concentrations were found in the less intensively managed peri-urban areas, where fertilization is not expected. Even in these sites, however, phosphorus availability remained adequate to support plant growth. Previous studies, such as Foti et al. (2021), have similarly reported an increase in phosphorus levels from rural to urban areas, interpreting this as a legacy of lawn fertilization practices in city parks.

Overall, no signs of problematic surface compaction were observed in the public green spaces sampled across Milan. Bulk density values in Milan's public green spaces were relatively low ($0.73\text{--}1.27\text{ g cm}^{-3}$, mean 1.03 g cm^{-3}), with even the highest recorded value falling within a range considered favourable for root growth ($<1.6\text{ g cm}^{-3}$; USDA, 2023) and not detrimental to soil biota ($<1.7\text{ g cm}^{-3}$; Beylich et al., 2010). These results are consistent with previous findings (Edmondson et al., 2011; Paradelo et al., 2025). In contrast, higher values have been reported in other studies. For example, in US cities, Scharenbroch et al. (2005) reported BD values of $1.4\text{--}1.7\text{ g cm}^{-3}$ in residential areas, while Pouyat et al. (2007) found an overall range of $0.7\text{--}1.7\text{ g cm}^{-3}$ across different urban uses. Similarly, Matziris et al. (2016) observed average values around 1.5 g cm^{-3} in urban parks in Thessaloniki, Greece.

Bulk density values significantly differed across green area categories, even when accounting for the effect of SOC, which was negatively correlated with BD, as expected. PPF showed the lowest mean BD (0.90 g cm^{-3}), significantly different from all the other categories. A key factor could be the presence of organic surface horizons (mainly OL and OF), which typically characterize forest soils even in relatively young woodlands. These organic layers play a crucial protective role for the underlying soil structure acting

as a physical buffer, to reduce the direct impact of trampling and mechanical loads, thereby limiting compaction. In addition, site management is minimal and foot traffic tends to be largely confined to designated paths. Among grassland categories, PP showed relatively low BD values, likely due to lower pedestrian traffic and low maintenance – typically limited to mowing – making them similar to semi-natural grassland systems. In contrast, UPC recorded the highest mean BD (1.15 g cm^{-3}), which was significantly greater than in peri-urban areas. This likely reflects the effects of intense and prolonged foot traffic, along with the more frequent use of maintenance machinery that characterize these areas. Despite the observed differences, all values remained within acceptable limits, confirming that soil physical conditions are generally suitable and do not indicate critical compaction. This general absence of critical compaction may be explained by the relatively high organic matter content, which improves soil structural stability (Leroy et al., 2008).

Not surprisingly, soil resistance to penetration profiles differed markedly between forested and grass-covered areas. In peri-urban forests, PR values were consistently low and increased gradually and almost linearly with depth. In contrast, grass-covered areas showed higher overall resistance, with a sharp rise in the top 10–15 cm, followed by either stable or more variable values at greater depths. This near-surface peak likely reflects compaction from trampling and maintenance machinery traffic, as commonly reported in urban soils (Gregory et al., 2006). These activities tend to compact the subsurface layers, while leaving the uppermost centimetres less dense due to biological activity, root growth, or recent surface works.

Differences in PR among green area categories were most evident in the topsoil, where use, management, and surface disturbance have the strongest impact. PR patterns were consistent with those observed for BD, highlighting the link between surface compaction and human activity. Below a depth of 10 cm, differences among categories became less pronounced. While a broad distinction between forested and grass-covered areas persisted, reflecting the influence of vegetation, deeper compaction appeared to reflect mainly site-specific disturbances rather than systematic differences across green area categories.

The ability of roots to overcome soil mechanical resistance varies across species and growth stages, but pressures up to approximately 2.5 MPa can be exerted by root tips in many crops (Gregory et al., 1994). Similar mean values have been observed in topsoil of urban prairies with no visible signs of compaction stress (Johnston et al., 2016), suggesting that PR values around this threshold are not uncommon in healthy soils. For trees, Sinnott et al. (2008) reported that about 90% of roots develop in soil volumes where penetration resistance remains below 3 MPa.

In our data, mean PR values by category remained below 2.5 MPa in the 0–10 cm layer. In deeper layers, UP and PP approached 2.5 MPa, while UGI and UPC exceeded it, reaching up to about 2.75 MPa. Forest soils consistently showed low PR across all depths. When considering the full dataset across all depths and

plots, PR ranged from 0.56 to 3.85 MPa. These values align with those reported in other European cities (Paradelo et al., 2025), although higher maximum values have been documented in comparable urban environments (Johnston et al., 2016). Taken together, these findings suggest that PR values observed in this study are generally not high enough to impair vegetation health. While some localized compaction may occur—particularly in the deeper layers of intensively used sites—the overall levels do not indicate critical constraints for root growth. Moreover, penetrometer readings tend to overestimate the actual resistance experienced by roots by a factor ranging from 2 to 8, according to various authors (Atwell, 1993; Gregory et al., 1994). Unlike roots, which can grow around obstacles or use fissures and biopores, the penetrometer is inserted vertically and is strongly affected by barriers such as stones or construction debris (Lampurlanés and Cantero-Martínez, 2003). In addition, root growth is not restricted to the vertical axis: many species extend laterally without negative effects on plant health (Hamza and Anderson, 2005).

To summarize, marked differences were observed between peri-urban and more central green spaces. In peri-urban sites, soils retained semi-natural features—such as low BD, sub-acidic pH, and moderate nutrient levels—consistent with their conception as parks reproducing elements of natural environments. Their current properties likely reflect also legacy effects: they were agricultural lands directly converted into public parks. In contrast, soils in more central sites showed clearer signs of alteration, including higher BD, elevated phosphorus levels, and pH values approaching neutrality or slight alkalinity. These areas have been exposed to sustained anthropogenic pressure over time, including trampling, surface reworking, and intensive ornamental management, and some of them represent the oldest green spaces in the city. Time plays a crucial role: the longer and more continuous the urban pressure, the more pronounced its imprint on soil properties (Scharenbroch et al., 2005). While these trends are consistent with patterns observed in other cities, soil conditions remain closely tied to site-specific land-use histories. Past uses, transformations, and management practices critically shape soil development, making local context essential for interpretation (Hazelton and Murphy, 2021).

2.5. Conclusions

The combined evaluation of physical and chemical properties indicates that the urban soils we investigated are, for the most part, not compacted, rich in organic matter, and not excessively altered by anthropogenic activities. Despite the urban context, artificial features were limited and key parameters remained within favourable ecological ranges. These findings suggest that soils in Milan's public green spaces maintain ecologically relevant properties that could support soil-based ecosystem services, providing further scientific evidence that urban soils are not uniformly or heavily degraded, as often assumed. Moreover, our work confirms that BD, PR, pH, and available P may serve as sensitive indicators of differentiated human impacts on surface soil characteristics in urban landscapes (Pouyat et al., 2007). Their variation reflects differences in management intensity, recreational pressure, vegetation cover, and location, and is most pronounced in the topsoil, where anthropogenic pressures are strongest. Taken together, these results may contribute to the development of a methodological framework that could be replicated in other urban contexts to guide soil assessment in public green spaces.

The generally good condition of urban soils in Milan highlights the importance of preserving and, where possible, improving this still undervalued component of urban ecosystems. Careful and site-specific management can help to maintain favourable physical and chemical properties, for instance by reducing unnecessary soil disturbance, limiting heavy machinery use, and promoting practices that sustain vegetation cover and organic matter content. In this regard, leaving leaf litter and mowed grass on site supports nutrient cycling, contributes to organic matter accumulation, and provides a physical buffer against soil compaction. Moreover, where fertilization is carried out, it should be preceded by soil analyses in order to avoid unnecessary phosphorus inputs in areas where its availability is already high. However, any soil protection strategy must be carefully balanced with the essential role of urban green spaces in providing opportunities for people to experience nature and to access cultural ecosystem services (Burghardt et al., 2015). For example, while restricting access in selected areas can help reduce surface compaction, such measures may not always be appropriate in public parks (Millward et al., 2011).

Overall, our findings may help foster greater recognition of the ecological role and value of urban soils in public green spaces, supporting their protection and informing more effective management decisions.

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Supplementary Materials

DESCRIPTIVE STATISTICS OF SOIL ORGANIC CARBON, TOTAL NITROGEN AND C:N RATIO BY GREEN AREA CATEGORY

Tab. S1. Mean \pm sd by green area categories. Layer: depth in cm. SOC = soil organic carbon, tN = total nitrogen, C:N = C:N ratio. PPf = peri-urban forest, PP = peri-urban park, UP = urban park, UGI = urban green island, UPC = urban park centre.

| | Layer | PPf | PP | UP | UGI | UPC |
|-----|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SOC | 0-10 | 3 \pm 0.62 | 3.23 \pm 0.83 | 3.38 \pm 0.91 | 3.25 \pm 0.81 | 3.26 \pm 0.54 |
| | (%) 10-20 | 1.66 \pm 0.65 | 1.73 \pm 0.56 | 1.59 \pm 0.77 | 1.77 \pm 0.69 | 2.04 \pm 0.39 |
| | 20-40 | 1.24 \pm 0.46 | 1.06 \pm 0.33 | 1.17 \pm 0.62 | 1.41 \pm 0.39 | 1.32 \pm 0.35 |
| tN | 0-10 | 0.27 \pm 0.06 | 0.3 \pm 0.08 | 0.32 \pm 0.08 | 0.28 \pm 0.09 | 0.29 \pm 0.04 |
| | (%) 10-20 | 0.17 \pm 0.07 | 0.17 \pm 0.04 | 0.15 \pm 0.06 | 0.16 \pm 0.06 | 0.18 \pm 0.03 |
| | 20-40 | 0.14 \pm 0.07 | 0.11 \pm 0.04 | 0.11 \pm 0.06 | 0.14 \pm 0.04 | 0.12 \pm 0.02 |
| C:N | 0-10 | 11 \pm 1 | 11 \pm 1 | 11 \pm 0 | 12 \pm 2 | 11 \pm 1 |
| | 10-20 | 10 \pm 1 | 10 \pm 2 | 10 \pm 1 | 12 \pm 2 | 11 \pm 1 |
| | 20-40 | 9 \pm 1 | 10 \pm 3 | 11 \pm 3 | 10 \pm 1 | 11 \pm 3 |

OUTPUT OF LINEAR MIXED MODELS WITH GREEN AREA CATEGORY AS THE ONLY FIXED EFFECT

Abbreviations: SOC = soil organic carbon, tN = total nitrogen, avP = available phosphorus, BD = bulk density, PR = penetration resistance; PPf = peri-urban forest, PP = peri-urban park, UP = urban park, UGI = urban green island, UPC = urban park centre. Numbers refers to the investigated soil layer: 1: 0-10 cm, 2: 10-20 cm, 3: 20-40 cm.

Model: log(avP1) ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|-----------|----|-----------|---------|
| (Intercept) | 3.0016787 | 0.1250780 | 30 | 23.998460 | 0.0000 |
| green_areaPPf | -0.1246034 | 0.2283601 | 30 | -0.545645 | 0.5893 |
| green_areaUGI | 0.8428891 | 0.2202225 | 25 | 3.827443 | 0.0008 |
| green_areaUP | 0.1087669 | 0.2202225 | 25 | 0.493895 | 0.6257 |
| green_areaUPC | 0.7587336 | 0.2202225 | 25 | 3.445305 | 0.0020 |

Random effects

| | (Intercept) | Residual |
|---------|--------------|-----------|
| StdDev: | 3.252303e-05 | 0.5731793 |

Model: log(pH1) ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|----------|---------|
| (Intercept) | 1.7787898 | 0.02976223 | 30 | 59.76668 | 0.0000 |
| green_areaPPf | -0.0447761 | 0.03711154 | 30 | -1.20653 | 0.2370 |
| green_areaUGI | 0.1298154 | 0.04385988 | 25 | 2.95977 | 0.0066 |
| green_areaUP | 0.0715626 | 0.04936372 | 25 | 1.44970 | 0.1596 |
| green_areaUPC | 0.1602757 | 0.04836870 | 25 | 3.31362 | 0.0028 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|------------|
| StdDev: | 0.06876265 | 0.07517103 |

Model: log(pH2) ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|----------|---------|
| (Intercept) | 1.7703061 | 0.03496674 | 30 | 50.62828 | 0.0000 |
| green_areaPPf | -0.1249699 | 0.04369234 | 30 | -2.86023 | 0.0076 |
| green_areaUGI | 0.1592466 | 0.05155218 | 25 | 3.08904 | 0.0049 |
| green_areaUP | 0.1269843 | 0.05800374 | 25 | 2.18924 | 0.0381 |
| green_areaUPC | 0.2140232 | 0.05684097 | 25 | 3.76530 | 0.0009 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|------------|
| StdDev: | 0.0806937 | 0.08853288 |

Model: log(pH3) ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|----------|---------|
| (Intercept) | 1.8196849 | 0.03986290 | 29 | 45.64859 | 0.0000 |
| green_areaPPf | -0.1308434 | 0.04077103 | 29 | -3.20922 | 0.0032 |
| green_areaUGI | 0.1309870 | 0.05843180 | 24 | 2.24171 | 0.0345 |
| green_areaUP | 0.1314415 | 0.06631853 | 24 | 1.98197 | 0.0590 |
| green_areaUPC | 0.2019454 | 0.06352951 | 24 | 3.17877 | 0.0040 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|------------|
| StdDev: | 0.09974703 | 0.08048326 |

Model: SOC1 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|-----------|-----------|----|-----------|---------|
| (Intercept) | 3.229627 | 0.2306580 | 30 | 14.001799 | 0.0000 |
| green_areaPPf | -0.079498 | 0.2829256 | 30 | -0.280986 | 0.7807 |
| green_areaUGI | 0.019827 | 0.3387734 | 25 | 0.058525 | 0.9538 |
| green_areaUP | 0.210181 | 0.3821745 | 25 | 0.549961 | 0.5872 |
| green_areaUPC | 0.034302 | 0.3741418 | 25 | 0.091681 | 0.9277 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|-----------|
| StdDev: | 0.5376283 | 0.5714884 |

Model: SOC2 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|-----------|----|-----------|---------|
| (Intercept) | 1.6782264 | 0.2133918 | 30 | 7.864532 | 0.0000 |
| green_areaPPf | 0.0599555 | 0.1519680 | 30 | 0.394527 | 0.6960 |
| green_areaUGI | 0.0930765 | 0.2946277 | 25 | 0.315912 | 0.7547 |
| green_areaUP | -0.0978174 | 0.3470342 | 25 | -0.281867 | 0.7804 |
| green_areaUPC | 0.3448535 | 0.3328795 | 25 | 1.035971 | 0.3101 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|----------|
| StdDev: | 0.5715838 | 0.293234 |

Model: SOC3 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|-----------|-----------|----|----------|---------|
| (Intercept) | 1.0383248 | 0.1270540 | 29 | 8.172309 | 0.0000 |
| green_areaPPf | 0.0980768 | 0.1539386 | 29 | 0.637116 | 0.5290 |
| green_areaUGI | 0.3699744 | 0.1915996 | 24 | 1.930977 | 0.0654 |
| green_areaUP | 0.1089851 | 0.2140921 | 24 | 0.509057 | 0.6154 |
| green_areaUPC | 0.3075121 | 0.2058026 | 24 | 1.494209 | 0.1482 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|-----------|
| StdDev: | 0.2980031 | 0.3103278 |

Model: N1 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|-------------|------------|----|-----------|---------|
| (Intercept) | 0.30276588 | 0.02192051 | 30 | 13.811988 | 0.0000 |
| green_areaPPf | -0.02226788 | 0.02719285 | 30 | -0.818887 | 0.4193 |
| green_areaUGI | -0.02192424 | 0.03226917 | 25 | -0.679418 | 0.5031 |
| green_areaUP | 0.01894849 | 0.03634545 | 25 | 0.521344 | 0.6067 |
| green_areaUPC | -0.00874561 | 0.03560296 | 25 | -0.245643 | 0.8080 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|------------|
| StdDev: | 0.05078823 | 0.05503143 |

Model: N2 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|-------------|------------|----|-----------|---------|
| (Intercept) | 0.17204321 | 0.01749555 | 30 | 9.833538 | 0.0000 |
| green_areaPPf | 0.00274600 | 0.01559602 | 30 | 0.176071 | 0.8614 |
| green_areaUGI | -0.01517391 | 0.02457002 | 25 | -0.617578 | 0.5424 |
| green_areaUP | -0.02096869 | 0.02859739 | 25 | -0.733238 | 0.4702 |
| green_areaUPC | 0.00856698 | 0.02760850 | 25 | 0.310302 | 0.7589 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|------------|
| StdDev: | 0.04526488 | 0.03044677 |

Model: N3 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|----------|---------|
| (Intercept) | 0.10896655 | 0.01286188 | 29 | 8.472054 | 0.0000 |
| green_areaPPf | 0.02085830 | 0.01645224 | 29 | 1.267809 | 0.2150 |
| green_areaUGI | 0.02954929 | 0.01962709 | 24 | 1.505535 | 0.1452 |
| green_areaUP | 0.00020809 | 0.02178597 | 24 | 0.009551 | 0.9925 |
| green_areaUPC | 0.01629098 | 0.02096747 | 24 | 0.776965 | 0.4448 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|------------|
| StdDev: | 0.02927982 | 0.03347904 |

Model: BD ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|----------|---------|
| (Intercept) | 0.9833333 | 0.02301895 | 30 | 42.71843 | 0.0000 |
| green_areaPPf | -0.0655556 | 0.04202666 | 30 | -1.55986 | 0.1293 |
| green_areaUGI | 0.0996667 | 0.04052904 | 25 | 2.45914 | 0.0212 |
| green_areaUP | 0.0946667 | 0.04052904 | 25 | 2.33577 | 0.0278 |
| green_areaUPC | 0.1636667 | 0.04052904 | 25 | 4.03826 | 0.0004 |

Random effects

| | (Intercept) | Residual |
|---------|--------------|-----------|
| StdDev: | 1.558415e-06 | 0.1054861 |

Model: PR1 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|-----------|---------|
| (Intercept) | 1.5713386 | 0.08890595 | 30 | 17.674166 | 0.0000 |
| green_areaPPf | -0.7744607 | 0.16231932 | 30 | -4.771217 | 0.0000 |
| green_areaUGI | 0.4697708 | 0.15653508 | 25 | 3.001057 | 0.0060 |
| green_areaUP | 0.2166399 | 0.15653508 | 25 | 1.383970 | 0.1786 |
| green_areaUPC | 0.3615096 | 0.15653508 | 25 | 2.309448 | 0.0295 |

Random effects

| | (Intercept) | Residual |
|---------|--------------|-----------|
| StdDev: | 1.733028e-05 | 0.4074183 |

Model: PR2 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|-----------|----|-----------|---------|
| (Intercept) | 2.3067505 | 0.1182910 | 29 | 19.500645 | 0.0000 |
| green_areaPPf | -0.9315066 | 0.2054271 | 29 | -4.534487 | 0.0001 |
| green_areaUGI | 0.4526275 | 0.1971702 | 25 | 2.295619 | 0.0304 |
| green_areaUP | 0.1266392 | 0.2023657 | 25 | 0.625794 | 0.5371 |
| green_areaUPC | 0.3167275 | 0.2021880 | 25 | 1.566500 | 0.1298 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|-----------|
| StdDev: | 0.1328409 | 0.4808185 |

Model: PR3 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|-----------|----|-----------|---------|
| (Intercept) | 2.5376232 | 0.1331558 | 21 | 19.057546 | 0.0000 |
| green_areaPPf | -0.8747987 | 0.2219264 | 21 | -3.941842 | 0.0007 |
| green_areaUGI | 0.2027507 | 0.2549740 | 20 | 0.795182 | 0.4358 |
| green_areaUP | -0.2749421 | 0.2549740 | 20 | -1.078314 | 0.2937 |
| green_areaUPC | 0.2023704 | 0.2219264 | 20 | 0.911881 | 0.3727 |

Random effects

| | (Intercept) | Residual |
|---------|-------------|-----------|
| StdDev: | 1.33332e-05 | 0.5326233 |

OUTPUT OF LINEAR MIXED MODELS WITH GREEN AREA CATEGORY AND ADDITIONAL SOIL COVARIATES AS FIXED EFFECTS

Models for BD and PR included additional covariates selected via stepwise procedure, in addition to the green area category. Abbreviations are as listed above.

Model: BD ~ SOC1 + green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|-----------|---------|
| (Intercept) | 1.1067915 | 0.06385002 | 29 | 17.334240 | 0.0000 |
| SOC1 | -0.0387144 | 0.01839575 | 29 | -2.104528 | 0.0441 |
| green_areaPPf | -0.0813048 | 0.04218361 | 29 | -1.927402 | 0.0638 |
| green_areaUGI | 0.1020091 | 0.04018413 | 25 | 2.538541 | 0.0177 |
| green_areaUP | 0.1011615 | 0.04104153 | 25 | 2.464858 | 0.0209 |
| green_areaUPC | 0.1668242 | 0.04091567 | 25 | 4.077269 | 0.0004 |

Random effects

| (Intercept) | Residual |
|--------------------|-----------|
| StdDev: 0.02263029 | 0.1005733 |

Model: PR1 ~ green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|------------|----|-----------|---------|
| (Intercept) | 1.5713386 | 0.08890595 | 30 | 17.674166 | 0.0000 |
| green_areaPPf | -0.7744607 | 0.16231932 | 30 | -4.771217 | 0.0000 |
| green_areaUGI | 0.4697708 | 0.15653508 | 25 | 3.001057 | 0.0060 |
| green_areaUP | 0.2166399 | 0.15653508 | 25 | 1.383970 | 0.1786 |
| green_areaUPC | 0.3615096 | 0.15653508 | 25 | 2.309448 | 0.0295 |

Random effects:

| (Intercept) | Residual |
|----------------------|-----------|
| StdDev: 1.733028e-05 | 0.4074183 |

Model: PR2 ~ SOC2 + Sand2 + Clay2 + green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|-----------|----|-----------|---------|
| (Intercept) | 2.0886830 | 0.4656049 | 26 | 4.485956 | 0.0001 |
| SOC2 | -0.1887195 | 0.1046543 | 26 | -1.803266 | 0.0829 |
| Sand2 | 0.0017384 | 0.0006937 | 26 | 2.505856 | 0.0188 |
| Clay2 | -0.0018820 | 0.0011508 | 26 | -1.635355 | 0.1140 |
| green_areaPPf | -1.0108985 | 0.1842116 | 26 | -5.487702 | 0.0000 |
| green_areaUGI | 0.4775097 | 0.1771392 | 25 | 2.695674 | 0.0124 |
| green_areaUP | 0.1605001 | 0.1817831 | 25 | 0.882921 | 0.3857 |
| green_areaUPC | 0.1852495 | 0.1888012 | 25 | 0.981188 | 0.3359 |

Random effects

| (Intercept) | Residual |
|----------------------|-----------|
| StdDev: 2.687395e-05 | 0.4515241 |

Model: PR3 ~ Sand3 + Clay3 + green_area + (1 | location)

Fixed effects

| | Value | Std.Error | DF | t-value | p-value |
|---------------|------------|-----------|----|-----------|---------|
| (Intercept) | 0.7419097 | 0.5196200 | 20 | 1.427793 | 0.1688 |
| Sand3 | 0.0029067 | 0.0008038 | 19 | 3.616338 | 0.0018 |
| Clay3 | 0.0028501 | 0.0014441 | 19 | 1.973533 | 0.0632 |
| green_areaPPf | -0.7980936 | 0.2015842 | 19 | -3.959107 | 0.0008 |
| green_areaUGI | 0.2186600 | 0.2326573 | 20 | 0.939837 | 0.3585 |
| green_areaUP | -0.0188891 | 0.2365085 | 20 | -0.079867 | 0.9371 |
| green_areaUPC | 0.2465615 | 0.2212860 | 20 | 1.114221 | 0.2784 |

Random effects:

| | (Intercept) | Residual |
|---------|--------------|-----------|
| StdDev: | 1.056586e-05 | 0.4709513 |

CHAPTER 3

EARTHWORM COMMUNITIES

Abstract

Urban soils can sustain diverse faunal communities despite intense human pressures. Among soil organisms, earthworms play key ecological roles, yet their responses to urbanisation remain poorly understood. In this study, earthworm communities in public green spaces in Milan were characterised in terms of abundance, biomass, and functional and taxonomic composition. The factors influencing these communities were investigated by considering soil properties (including heavy metal concentrations), vegetation cover, land use, and landscape configuration. The study was conducted at a subset of 15 sites selected from those investigated in Chapter 2.

Soils in Milan's green spaces supported high earthworm abundance and biomass (mean \pm SD: 428 ± 256 ind m^{-2} and 128.4 ± 83.0 g m^{-2} , respectively), together with high taxonomic richness (mean \pm SD: 7.5 ± 2 taxa per site), compared with other urban environments. Pb concentration and patch area emerged as the main drivers of earthworm community patterns. Increasing Pb levels, reflecting long-term urban inputs, were associated with reduced biomass and taxonomic richness and shifts in functional composition, with anecic earthworms being particularly sensitive and endogeics being less affected. Patch area reflected a broader urban–peri-urban gradient of human pressure across the study sites, rather than direct ecological connectivity effects.

Clear contrasts in the relative abundance of earthworm ecological categories were observed between urban and peri-urban green spaces. Endogeic earthworms predominated in urban contexts, whereas anecic earthworms were more abundant in peri-urban parks, particularly in forested areas. Epigeic earthworms, although rare overall, occurred mainly in peri-urban parks. Earthworm species composition varied along a main environmental gradient primarily related to soil pH, bulk density and soil organic carbon, with most taxa showing weak responses to the gradient and a few species departing from the central pattern.

To our knowledge, this study provides the first systematic dataset on earthworm communities in Italian cities and advances current understanding of urban soil biodiversity.

3.1 Introduction

Urban ecosystems, despite intense human pressures, can sustain diverse and locally abundant faunal communities (Schmidt, 2024). Yet, while the effects of urbanization on aboveground taxa such as birds and mammals have been widely explored (McCleery, 2010; Shochat et al., 2010; Boakes et al., 2024), belowground biodiversity remains comparatively understudied (Guilland et al., 2018). Despite this gap of knowledge, urban soils and the organisms they host are recognised as playing a crucial role in maintaining key ecosystem services that support city sustainability and human well-being (Ziter, 2016; Schwarz et al., 2017; Weiskopf et al., 2024).

Among soil organisms, earthworms are particularly important. They strongly influence soil structure and functioning through bioturbation, actively modifying their environment, and acting as “soil ecosystem engineers” (Jones et al., 1994; Bartlett et al., 2010; Lavelle et al., 2016). They provide multiple ecosystem services, such as soil structure formation, nutrient cycling, water regulation, and play a key role in urban food webs (Blouin et al., 2013), thus being regarded as keystone organisms.

Despite their ecological importance, the effects of urban environments on earthworm communities remain poorly understood (Guilland et al., 2018). In a recent global meta-analysis, Phillips et al. (2025) reported substantial gaps in data describing earthworm communities in urban environments, including their temporal dynamics, and a lack of consistent information on the drivers underlying observed patterns. Addressing these gaps is essential to improve our understanding of urban soil biodiversity and to support evidence-based conservation and management strategies at the city scale (Eydoux et al., 2024).

Urban soils display a high degree of heterogeneity, and within the same city it is often possible to find markedly different conditions —from heavily modified environments to relatively undisturbed or semi-natural soils (De Kimpe and Morel, 2000; Pouyat et al., 2007; Morel et al., 2015). This strong spatial variability, driven by differences in land use, vegetation cover, management practices, and historical legacies (Pickett and Cadenasso, 2009; Ziter and Turner, 2018) creates a mosaic of different habitat conditions. Earthworm communities often mirror this spatial heterogeneity, showing contrasting patterns in abundance and biomass across urban sites (Schmidt, 2024), ranging from abundant populations with biomass and densities comparable to non-urban or semi-natural reference sites (Phillips et al., 2025; Francini et al., 2018) to low abundance and biomass in more disturbed urban soils (Li et al., 2020).

More than simply affecting abundance and biomass, urbanization can also alter earthworm community structure, providing diagnostically informative patterns (Phillips et al., 2025). Across urban contexts, assemblages may range from relatively diverse species compositions under locally favourable soil and habitat conditions to functionally simplified and biotically homogenized communities under stronger

urban pressures (McKinney, 2006; Pouyat et al., 2010). At the functional level, communities may become dominated by a single ecological category, a pattern reported in urban soils, including engineered soils (Maréchal et al., 2021). At the species level, urban earthworm assemblages may be dominated by a limited number of widespread generalist taxa (Eydoux et al., 2024) and may show increasing similarity among different urban contexts, even across diverse bioclimatic regions (Tóth et al., 2020). Although the introduction of exotic earthworm species can represent an ecological concern (Hendrix et al., 2008), their occurrence in European urban environments appears to be limited (Phillips et al., 2025).

While at the global scale urban earthworm communities are primarily shaped by climatic conditions, at the local scale they are structured by multiple interacting drivers (Phillips et al., 2025). These include landscape features and vegetation cover (Tóth et al., 2020), site age (Maréchal et al., 2021), and soil physical and chemical properties such as organic carbon, moisture, pH, texture, and compaction (Smetak et al., 2007; Xie et al., 2018). Soil contamination, including heavy metals, has also been identified as a potential influencing factor, although its effects on urban earthworm communities remain poorly understood (Chatelain et al., 2024). Moreover, urban management practices, such as mowing, irrigation, and fertilization, can further influence earthworm communities (Eydoux et al., 2024). In addition, it should be considered that urban earthworm communities are shaped not only by current environmental conditions but also by site-specific legacies of land use and management, which condition interactions among drivers (Guilland et al., 2018). This highlights the importance of accounting for local context when interpreting patterns of earthworm communities influenced by urban constraints.

From this perspective, we investigated how urban pressures and site-specific environmental conditions interact to shape earthworm communities in urban soils, focusing on Milan, a medium-sized European city. While several components of urban fauna in Milan, such as birds and mammals, have been previously investigated (Canedoli et al., 2018; Dondina et al., 2025), soil fauna has not been assessed to date.

Here, we address this knowledge gap by pursuing two main objectives. First, we quantified earthworm biodiversity in urban green spaces, providing the first dataset on earthworm communities in Italian cities. Second, we evaluated the factors shaping earthworm communities, considering soil properties (including heavy metal concentrations), vegetation cover, and landscape configuration. To this end, we considered a range of green space categories—including urban and peri-urban parks (both grassland and forested areas) and small urban green islands—selected to capture contrasting conditions in vegetation structure, management intensity, land-use history, and urban context.

3.2 Materials and methods

3.2.1 Study area and sampling design

The study was conducted in Milan (Northern Italy). The city is located in the Po Valley alluvial plain and is characterized by a predominantly flat landscape, with an average elevation of approximately 100 m a.s.l. The climate is continental, with a mean annual temperature of 13.0 °C and a mean annual precipitation of 920 mm. Monthly mean temperatures range from 2.3 °C in January to 23.8 °C in July (1991–2021).

Its long history of human activity has profoundly shaped the territory; the present-day city displays a clear urban gradient, from the compact historical core to more heterogeneous peripheral areas where residential, industrial, and semi-natural zones coexist. With over one million inhabitants, Milan represents one of the largest and most densely populated regions in the country. Public green spaces cover about 25 km², roughly 14 % of the municipal area (Municipality of Milan, 2024).

The research focused on public green spaces across the city. Sampling sites were selected from those investigated in Chapter 2, which analyzed the chemical and physical properties of Milan's soils and provided the background for the present study. A subset of 15 sites was selected for the earthworm survey (Fig. 1). Sites were chosen to represent the five categories of urban green areas defined in Chapter 2, based on vegetation cover (grassland and forest), green space type, and levels of public use typically observed throughout the city. The five categories considered were grasslands in urban parks located inside and outside the historic centre (Urban Park Center – UPC and Urban Park – UP, respectively), grasslands in small urban green islands (UGI), grasslands in peri-urban parks (PP), and forested areas within peri-urban parks (PPf). Three sites were chosen within each category, avoiding plots showing extreme values of pH or soil organic carbon and ensuring that the selected sites spanned the range of bulk density values observed for that category. Overall, this design aimed to capture the diversity of vegetation cover, management intensity, and land-use history across Milan's green spaces.

Urban parks inside (UPC) and outside (UP) the historic centre, together with urban green islands (UGI), are intensively managed green areas characterized by frequent mowing, irrigation, and occasional chemical fertilization. UPC are historic sites subjected to long-term recreational use, with heavy foot traffic and frequent disturbance, whereas UP are generally more recent and less frequented. UGI are small, vegetated spaces, such as green areas within squares or traffic islands, fully enclosed by the urban matrix and often exposed to strong local disturbances due to their limited size and proximity to roads and buildings. Peri-urban parks are extensive green areas near the city boundary, including grassland (PP) and forest (PPf) patches. They are managed with low intensity (occasional mowing, no irrigation or fertilization) and are exposed to reduced human pressure. These areas often retain semi-natural features

and maintain some degree of ecological connection with the surrounding landscape. All green spaces considered in this study are publicly accessible and not subject to trampling restrictions.

For a detailed description of the study area, the categories of urban green areas, and other contextual information, see Chapter 2.



Figure 1. Location of the selected sampling sites ($n=15$). Sites are grouped by green area category, as indicated in the legend; numbers identify individual sampling sites within each category.

3.2.2 Data collection

3.2.2.1 Earthworm sampling, identification and community metrics

Earthworm sampling was conducted in October–November 2024, one of the two main periods of peak activity for temperate earthworm communities (ISO 23611-6). In each study site, sampling took place within the 4×4 m plot previously defined in Chapter 2. Four soil blocks (25×25 cm, 20 cm depth) were extracted per plot and hand-sorted to collect earthworms (Fig. 2). The litter layer was also inspected for the presence of earthworms. Specimens were then preserved in 70% ethanol (ISO 23611-1). Sampling order was arranged to minimize potential biases related to seasonal progression and weather variability: one site per green area category was sampled in rotation before proceeding to the next round.

In the laboratory, earthworms were counted, individually weighed (fresh weight with gut content), associated with a stage of development (juvenile, sub-adult and adult), identified to species or subspecies level when possible, and assigned to their ecological category: epigeic, endogeic and anecic (Bouché, 1972). Juveniles identified only to the genus level were redistributed among species or subspecies in

proportion to the number of identified subadults and adults of the same genus and ecological category within each plot. When no adults or subadults were present, juveniles were kept at the genus level. In addition, a few unidentified individuals showing distinct morphological traits were treated as separate morpho-taxa.

Data from the four soil blocks were pooled, and all earthworm metrics were calculated at the plot level. Abundance (individuals m^{-2}) and biomass (g m^{-2}) were estimated for each plot by multiplying field data by 16, to obtain an estimation per square metre. Taxa richness (number of taxa per plot), Shannon's diversity index (H'), and Pielou's evenness (J') were calculated for each plot based on all identified taxa. Relative abundance of epigeic, endogeic, and anecic groups was calculated for each plot.

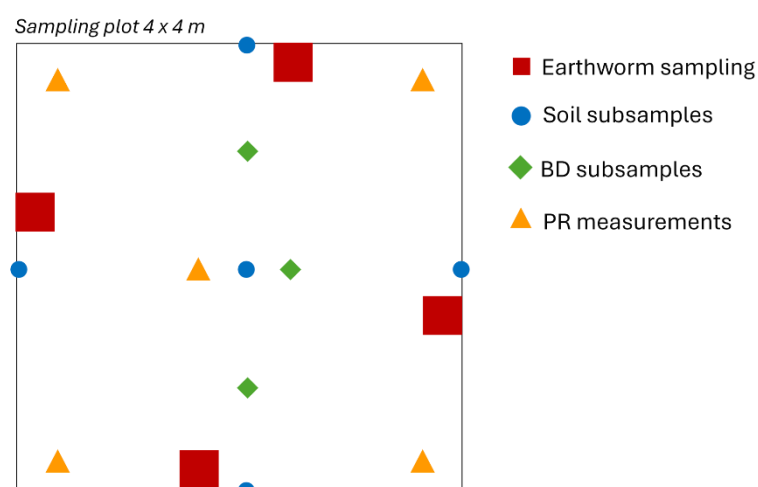


Figure 2. Schematic layout of the sampling plot. Symbols indicate the spatial locations of different measurements within each plot.

3.2.2.2 Characterization of soil chemical and physical properties

This study used the dataset on soil chemical and physical properties collected in the previous chapter. From the three soil layers sampled in Chapter 2, only data from the 0–10 and 10–20 cm layers were used, as they correspond to the depth sampled for earthworms. All methodological details are provided in Chapter 2; in brief, composite soil samples (five subsamples per plot; Fig. 2) were air-dried, sieved (< 2 mm), and analyzed for pH (soil to water ratio of 1:2.5), soil organic carbon (SOC, after carbonate removal), total nitrogen content, available phosphorus (Olsen method), and particle-size distribution (sieving and sedimentation). In addition, pseudo-total concentrations of heavy metals (Cr, Cu, Ni, Pb, Zn) were determined specifically for this biological part of the study. Analysis were done on the same soil samples previously collected from the 0–10 and 10–20 cm layers, by extraction with a solution of HCl 37% and HNO₃ 65% in a microwave oven and determined through atomic absorption spectroscopy (AAS).

Bulk density (BD) was measured in the top 5 cm from three undisturbed soil cores (100 cm³ each; Fig. 2), combined into a composite sample. Soil penetration resistance (PR) was measured in the field at 2.5 cm

intervals using a penetrometer, with five repeated readings averaged per plot (Fig. 2). To allow direct comparison among measurements taken under different soil moisture conditions, PR data were corrected for water content, adjusting values to a common reference soil water content corresponding to field capacity (see Chapter 2 for methodological details).

3.2.2.3 Environmental variables

At each site, soil temperature and soil water content (Time Domain Reflectometry, TDR) were measured concurrently with earthworm sampling at two depths (0–5 and 10–15 cm). Measurements were taken in triplicate and averaged to obtain one value per depth and site.

In addition to these local soil variables, spatial variables were derived from GIS data layers using QGIS (v. 3.16.11). The distance of each plot from the city center (Piazza Duomo) was calculated as a proxy for the urbanization gradient, used as a surrogate measure integrating multiple urban pressures for which no single, spatially explicit indicator is currently available.

Patch area was defined as the surface of the continuous green space surrounding each sampling plot. Patches were delineated in QGIS from aerial orthophotos, considering as interruptions impervious surfaces (concrete, asphalt) and wide semi-pervious features (e.g., compacted soil or gravel roads). Narrow semi-pervious pedestrian paths (3–4 m wide) within green areas were not considered barriers, while broader semi-pervious surfaces (> 6–7 m wide) or especially paved areas were treated as boundaries, as they likely reduce earthworm dispersal through increased dryness, exposure, traffic-related mortality or physical discontinuities such as curbs (Dupont et al., 2017; Maréchal et al., 2024). Patch area was used as an indicator of local habitat size and potential ecological connectivity for earthworm communities within the urban matrix, under the assumption that smaller and more isolated green patches may offer reduced opportunities for dispersal and recolonisation following disturbance.

3.2.3 Statistical analyses

Analyses were based on soil chemical and physical data representative of the 0–20 cm soil layer sampled for earthworms. Soil properties measured separately in the 0–10 and 10–20 cm layer were averaged to obtain a single value per site, while penetration resistance values were averaged across the same depth interval. Bulk density, measured in the 0–5 cm layer, was retained as measured. Soil temperature and volumetric water content, recorded at two depths, were averaged to obtain one representative site-level value.

Basic descriptive statistics (mean, minimum, maximum, standard deviation) were computed across the 15 sites for earthworm, soil and environmental variables.

To explore the relationships between earthworm communities and soil properties, vegetation cover and environmental variables, linear models (LMs) were fitted.

Before model fitting, one-way ANOVAs were performed to test whether earthworm community attributes differed across the five green area categories (UPC, UP, UGI, PP, and PPf). This exploratory step was intended to assess the potential relevance of this categorical factor for subsequent analyses. Residuals were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test), and post-hoc pairwise comparisons were conducted using Tukey’s HSD test ($p < 0.05$).

The continuous covariates considered as candidates for inclusion in the linear models were checked for pairwise Pearson correlations, and for each pair with $|r| > 0.7$, one variable was excluded to avoid collinearity (Dormann et al., 2013). Given the limited number of observations ($n = 15$), predictors were selected based on their ecological relevance to earthworm communities, with the aim of keeping model complexity low. An initial set of predictors for modelling was thus defined. The best model for each response variable was then selected using a stepwise procedure based on the Akaike Information Criterion corrected for small samples (AICc), applied to the initial set of predictors. The significance of predictors in the final models was assessed by Type II ANOVA. Model assumptions were checked by visual inspection of diagnostic plots and by applying the Shapiro–Wilk test for normality and the Breusch–Pagan test for homoscedasticity. Residual spatial autocorrelation was tested using Moran’s I across all fitted models. All continuous predictors were standardized prior to analysis.

To assess how earthworm community composition varied in relation to environmental factors, a Redundancy Analysis (RDA) was performed using Hellinger-transformed taxa abundance data (Legendre and Gallagher, 2001). Rare taxa occurring in only one or two sites and representing less than 5 % of site-level abundance were excluded from the analysis to reduce noise and improve analysis stability. The same set of variables used in the linear models was included as explanatory variables; continuous variables were standardized prior to analysis. The significance of the overall RDA model, of individual canonical axes, and of each explanatory variable was tested by permutation tests (999 permutations). RDA results were interpreted based on the first two canonical axes, which represented the main environmental gradients shaping species composition.

All statistical analyses were performed in R (version 4.5.0, R Core Team 2025) using the *stats*, *spdep*, *lmtree*, *car*, *emmeans*, *MuMIn* and *vegan* packages.

3.3 Results

3.3.1 Chemical and physical properties of soils

Soil physical and chemical properties in the 15 plots (Table 1) reflected the overall patterns previously described in Chapter 2. Soil texture classes ranged from loam to sandy loam across all sites. Bulk density and penetration resistance covered a wide range of values, with BD ranging from 0.85 to 1.27 g cm⁻³ and PR from 0.85 to 2.92 MPa. The lowest BD and PR values were observed in peri-urban forest sites, whereas higher BD values occurred in parks located in the historical city centre (UPC), and higher PR values were more generally associated with urban contexts (UP, UPC, UGI). Organic carbon content varied from 1.51 to 4.04% (mean ± SD: 2.37 ± 0.68%) and showed no clear spatial pattern across urban and peri-urban sites. The C:N ratio exhibited a relatively narrow range (9–12). Soil pH ranged from 4.7 to 7.5, with more acidic conditions in peri-urban parks and generally higher pH values in urban green areas. Available phosphorus showed wide variability across sites (mean ± SD: 26 ± 15 mg kg⁻¹).

Table 1. Soil chemical and physical properties of the study sites. All soil properties refer to the 0–20 cm layer, except BD (0–5 cm layer). avP: available phosphorus. Concentrations of other heavy metals are reported in Table S1.

| Plot | Text | Clay g kg ⁻¹ | BD g cm ⁻³ | PR MPa | pH | SOC % | C:N | avP mg kg ⁻¹ | Pb mg kg ⁻¹ |
|-------|------|----------------------------|--------------------------|-----------|-----|----------|-----|----------------------------|---------------------------|
| PPf-1 | SL | 114 | 0.85 | 1.10 | 5.1 | 1.57 | 11 | 16 | 106 |
| PPf-2 | L | 110 | 0.85 | 0.85 | 4.7 | 1.90 | 11 | 18 | 167 |
| PPf-3 | L | 107 | 0.91 | 0.99 | 5.3 | 2.79 | 10 | 15 | 86 |
| PP-1 | L | 120 | 1.11 | 2.06 | 5.0 | 2.03 | 10 | 12 | 177 |
| PP-2 | SL | 83 | 1.17 | 1.61 | 5.7 | 1.51 | 9 | 9 | 54 |
| PP-3 | SL | 68 | 0.98 | 1.93 | 5.4 | 2.56 | 11 | 20 | 77 |
| UP-1 | SL | 82 | 1.02 | 2.37 | 5.7 | 2.03 | 10 | 9 | 66 |
| UP-2 | L | 81 | 0.99 | 2.01 | 6.2 | 4.04 | 11 | 67 | 270 |
| UP-3 | SL | 101 | 1.03 | 2.47 | 7.1 | 2.75 | 10 | 22 | 176 |
| UGI-1 | L | 152 | 1.13 | 2.45 | 7.4 | 1.67 | 11 | 36 | 55 |
| UGI-2 | SL | 92 | 1.05 | 2.07 | 7.5 | 2.82 | 12 | 41 | 125 |
| UGI-3 | L | 87 | 1.17 | 2.92 | 7.3 | 2.01 | 11 | 26 | 88 |
| UPC-1 | SL | 60 | 1.24 | 2.17 | 7.0 | 2.64 | 11 | 39 | 246 |
| UPC-2 | SL | 76 | 1.27 | 2.45 | 7.5 | 2.13 | 11 | 26 | 126 |
| UPC-3 | SL | 59 | 1.16 | 2.60 | 7.2 | 3.14 | 11 | 28 | 334 |
| | Min | 59 | 0.85 | 0.85 | 4.7 | 1.51 | 9 | 9 | 54 |
| | Max | 152 | 1.27 | 2.92 | 7.5 | 4.04 | 12 | 67 | 334 |
| | Mean | 93 | 1.06 | 2.00 | 6.2 | 2.37 | 11 | 26 | 144 |
| | SD | 25 | 0.13 | 0.62 | 1.0 | 0.68 | 1 | 15 | 85 |

Heavy metal concentrations varied substantially across sites (Table 1; see also Tab. S1), with broadly consistent patterns among the analyzed elements. In general, concentrations tended to be lower in peri-urban parks, while higher values were observed in urban parks. Especially for Pb, urban green islands generally exhibited concentrations comparable to those observed in peri-urban parks. Among the analyzed metals, Pb displayed the highest concentrations (mean \pm SD: 144 ± 85 mg kg⁻¹), reaching the maximum value of 334 mg kg⁻¹. It is essential to note that the heavy metal concentrations reported here refer to the fine-earth fraction (<2 mm) of the surface layer (0–10 cm). They are not directly comparable with the threshold values established by the Italian Legislative Decree 152/2006, which require concentrations averaged over the entire soil profile (0–100 cm) and expressed on both fine-earth and gravel fractions. Therefore, any exceedances of regulatory limits in the fine fraction of the surface horizon do not constitute evidence of contamination under the law; rather, they may indicate the opportunity for further investigation in accordance with standardized protocols consistent with regulatory requirements.

3.3.2 Environmental characteristics

During sampling, mean soil temperature across sites ranged from 8.2 to 18.6 °C (mean = 13.2 °C), and soil water content from 18% to 32% (mean = 26%). The distance of the plots from the city center (Piazza Duomo) ranged from approximately 1–1.5 km for parks in the historical center to 8–9 km for peri-urban parks. Patch area varied widely, from 0.2 ha to 18 ha, and in general tended to increase with distance from the city center, although this pattern was not consistent across all sites (Table 2).

Table 2. Environmental variables across the 15 plots. Dist. Center: distance from the city center (km); T: soil temperature (°C); VWC: soil Volumetric Water Content (%); Patch area (ha).

| Plot | Dist. Center | Patch area | T | VWC |
|-------|--------------|------------|------|-----|
| PPf-1 | 9.1 | 18.0 | 9.2 | 20 |
| PPf-2 | 8.8 | 17.0 | 9.4 | 18 |
| PPf-3 | 7.8 | 4.5 | 17.6 | 29 |
| PP-1 | 8.5 | 15.0 | 10.1 | 27 |
| PP-2 | 7.1 | 3.0 | 10.5 | 30 |
| PP-3 | 8.5 | 7.0 | 17.9 | 24 |
| UP-1 | 5.4 | 0.6 | 18.6 | 32 |
| UP-2 | 1.9 | 0.2 | 8.9 | 30 |
| UP-3 | 5.4 | 1.2 | 13.8 | 19 |
| UGI-1 | 2.4 | 0.3 | 9.4 | 28 |
| UGI-2 | 3.3 | 0.7 | 18.6 | 20 |
| UGI-3 | 2.5 | 0.2 | 17.9 | 28 |
| UPC-1 | 1.3 | 0.3 | 16.9 | 31 |
| UPC-2 | 1.6 | 1.2 | 8.2 | 25 |
| UPC-3 | 1.1 | 0.2 | 11.2 | 27 |
| Min | 1.1 | 0.2 | 8.2 | 18 |
| Max | 9.1 | 18.0 | 18.6 | 32 |
| Mean | 5.0 | 4.6 | 13.2 | 26 |
| SD | 3.1 | 6.5 | 4.2 | 5 |

3.3.3 Earthworm community characteristics

Considering all 15 sites, earthworm abundance averaged 428 ± 256 ind m^{-2} (range = 68–1104 ind m^{-2}), and biomass 128.4 ± 83.0 g m^{-2} (range = 26.1–332.8 g m^{-2}) (Table 3). Juveniles accounted for 59% of all individuals, while subadults and adults represented 9% and 31%, respectively. In contrast, adults contributed the largest share of total biomass (54%), followed by juveniles (34%) and subadults (13%). Data on developmental stages are reported also in Supplementary Material, Table S2. Considering ecological categories across all sites, anecic earthworms represented 46% of total abundance and 71% of total biomass, endogeic 52% and 28%, and epigeic 2% and 1%, respectively.

Table 3. Earthworm abundance and biomass across the study sites, reported as total values and by ecological category.

| Plot | Earthworm abundance (ind m^{-2}) | | | | Earthworm biomass (g m^{-2}) | | | |
|-------|-------------------------------------|--------|----------|---------|---------------------------------|--------|----------|---------|
| | Total | Anecic | Endogeic | Epigeic | Total | Anecic | Endogeic | Epigeic |
| PPf-1 | 192 | 128 | 60 | 4 | 55.9 | 51.1 | 4.8 | 0.1 |
| PPf-2 | 192 | 116 | 56 | 20 | 74.5 | 65.1 | 8.8 | 0.7 |
| PPf-3 | 508 | 412 | 96 | 0 | 231.3 | 212.7 | 18.7 | 0.0 |
| PP-1 | 68 | 20 | 12 | 36 | 26.1 | 16.4 | 3.3 | 11.1 |
| PP-2 | 396 | 232 | 136 | 28 | 159.2 | 133.5 | 24.1 | 1.6 |
| PP-3 | 840 | 576 | 260 | 4 | 332.8 | 299.4 | 32.6 | 0.0 |
| UP-1 | 352 | 108 | 240 | 4 | 108.1 | 62.6 | 45.4 | 0.1 |
| UP-2 | 396 | 76 | 320 | 0 | 119.5 | 49.3 | 70.2 | 0.0 |
| UP-3 | 316 | 72 | 244 | 0 | 120.5 | 51.5 | 69.0 | 0.0 |
| UGI-1 | 1104 | 620 | 468 | 16 | 253.4 | 211.6 | 38.9 | 2.9 |
| UGI-2 | 320 | 88 | 232 | 0 | 79.0 | 38.5 | 40.5 | 0.0 |
| UGI-3 | 448 | 136 | 300 | 12 | 104.0 | 67.7 | 35.4 | 0.9 |
| UPC-1 | 380 | 112 | 268 | 0 | 80.3 | 31.4 | 48.9 | 0.0 |
| UPC-2 | 504 | 252 | 248 | 0 | 99.0 | 67.9 | 29.0 | 0.0 |
| UPC-3 | 400 | 20 | 380 | 0 | 81.9 | 15.3 | 66.6 | 0.0 |
| Min | 68 | 20 | 12 | 0.0 | 26.1 | 15.3 | 3.3 | 0.0 |
| Max | 1104 | 620 | 468 | 36.0 | 332.8 | 299.4 | 70.2 | 11.1 |
| Mean | 428 | 198 | 221 | 8.3 | 128.4 | 91.6 | 35.7 | 1.2 |
| SD | 256 | 190 | 127 | 11.7 | 83.0 | 84.4 | 22.0 | 2.9 |

In most sites, juveniles were numerically dominant, although adults prevailed in several cases, and subadults were consistently scarce (Fig. 3a). Peaks in total abundance were largely driven by high numbers of juveniles. Biomass was dominated by adults at nearly all sites, whereas juveniles and subadults contributed comparatively little (Fig. 3b).

Both earthworm abundance and biomass varied widely across sites (Fig 3a , 3b; Table 3), with no significant differences among green area categories. Site-level values indicated pronounced internal variability within peri-urban parks (PP and PPf) and urban green islands for both metrics, whereas urban parks (UP and UPC) showed lower variability. Mean (\pm SD) of earthworm abundance and biomass (total and by ecological categories) across green area categories are provided in Supplementary Materials, Table S3).

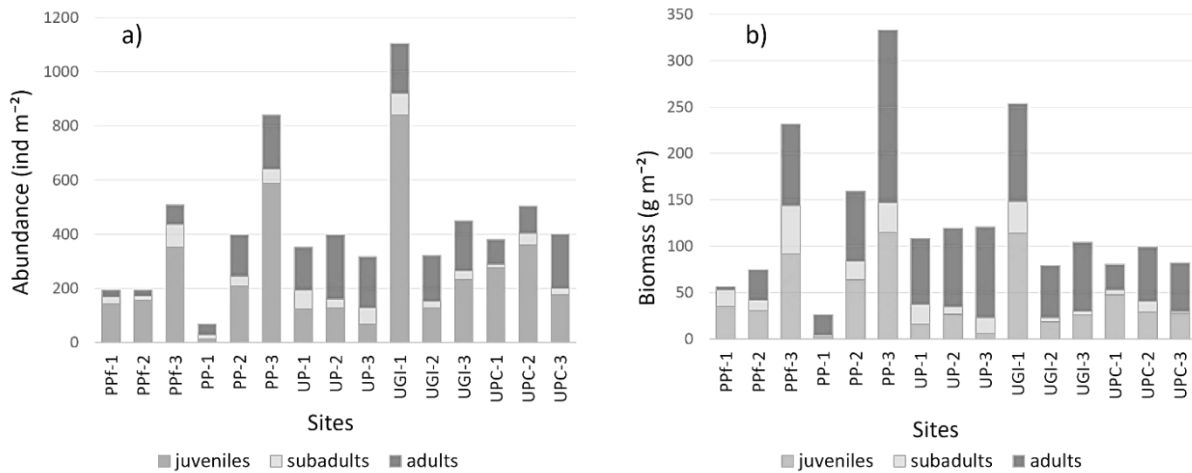


Figure 3. Earthworm (a) abundance and (b) biomass across the study sites, divided by developmental stage.

The functional composition of earthworm communities, expressed as the relative abundance of ecological categories, varied considerably among sites, showing clear contrasts between urban and peri-urban areas (Fig. 4). Anecic earthworms accounted for the highest proportions in most peri-urban parks and forests (PP, PPf), except for one site dominated by epigeic. Endogeic prevailed in all urban sites (UP, UPC, UGI), with only two exceptions. Epigeic were generally rare, being almost completely absent from UP and UPC and occurring at slightly higher relative abundances in PP and PPf. These overall patterns were confirmed by the one-way ANOVA. Of all ANOVA results, only the analysis of relative abundances, as well as endogeic abundance and biomass, showed differences among green area categories. ($p < 0.05$; see SM).

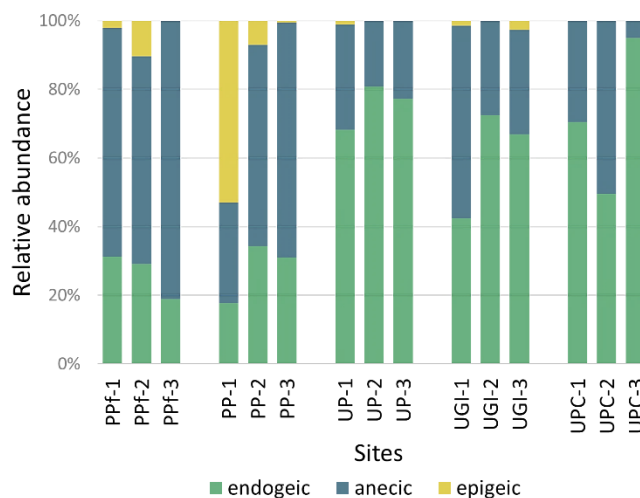


Figure 4. Relative abundance (%) of earthworm ecological categories across the study sites.

A total of 18 taxa were recorded across the 15 sites: 13 identified to species or subspecies level, 1 only to genus level, and 4 distinguished as morpho-taxa (Table 4). All taxa were assigned to an ecological category, except for a single exotic species.

Table 4. List of earthworm taxa recorded at the study sites, grouped by ecological category. The exotic species *Pheretima diffringens* could not be assigned to an ecological category.

| | | |
|----------|---------------|---|
| Endogeic | ACCT | <i>Allolobophora chlorotica</i> |
| | ARR | <i>Aporrectodea rosea</i> |
| | KG | <i>Koinodrillus georgi</i> |
| | NCCT | <i>Aporrectodea caliginosa caliginosa</i> |
| | OTL | <i>Octolasion tyrtaeum lacteum</i> |
| | Mt1 | Morpho-taxa 1 |
| | Mt2 | Morpho-taxa 2 |
| Mt3 | Morpho-taxa 3 | |
| Anecic | LT | <i>Lumbricus terrestris</i> |
| | NCM | <i>Aporrectodea caliginosa meridionalis</i> |
| | NG | <i>Aporrectodea giardi</i> |
| | NLL | <i>Aporrectodea longa</i> |
| | NN | <i>Aporrectodea nocturna</i> |
| | OcTr | <i>Octodrilus transpadanus</i> |
| Epigeic | DX | <i>Dendrobaena</i> (genus) |
| | LC | <i>Lumbricus castaneus</i> |
| | Mt4 | Morpho-taxa 4 |
| | PD | <i>Pheretima diffringens</i> (exotic) |

The most frequent and abundant species were 2 anecic species and 3 endogeic species (Table 5; see also Tables S4 and S5), which were recorded at most or all sites (12–15 of 15). Within the anecics, *Aporrectodea nocturna* was the most frequently recorded species, showing wide variation in relative abundance across sites (1–75%). Its dominance was mainly observed in peri-urban parks (PP and PPf), whereas it was generally less abundant in urban green areas. *A. caliginosa meridionalis* was also widespread but highly variable, representing from 0% (in 3 plots) to 39% of the community; in contrast to *A. nocturna*, it tended to be less represented in peri-urban parks than in urban areas.

Endogeic species also showed pronounced variability in relative abundance. *Allolobophora chlorotica* was one of the most frequent species, often reaching high relative abundances (up to ~55%), and was absent in only two plots; it was more prevalent in urban than in peri-urban green spaces. *Aporrectodea caliginosa caliginosa* was frequent across sites, ranging from 0% (in one plot) to 30% of total abundance and did not display a clear pattern among green area types. *A. rosea* ranged from 0% (in two plots) to 28% of total

abundance and was more common in urban green islands (UGI) and urban parks in the city centre (UPC). Epigeic taxa were few overall, with only three taxa recorded (Table 5). Among them, *Lumbricus castaneus* was the most regularly occurring species, reaching locally high relative abundances at one site (53%). These widespread species formed the core of the community.

A few comparatively rare taxa with more restricted geographic distributions were also recorded (Table 5), including *Koinodrillus georgi*, which was found at a single site where it accounted for about 27% of individuals, and *Octodrilus transpadanus*, recorded at three sites with relative abundances ranging from 3% to nearly 30%. One exotic species, *Pheretima diffringens*, was represented by a single individual.

Table 5. Relative abundance of earthworm taxa (%) across the study sites. Taxon codes are reported in Table 4. LAX and OX refer to genus-level identifications of *Lumbricus* (anecic) and *Octolasion*, respectively, used when identification to species level was not possible.

| | | PPf-1 | PPf-2 | PPf-3 | PP-1 | PP-2 | PP-3 | UP-1 | UP-2 | UP-3 | UGI-1 | UGI-2 | UGI-3 | UPC-1 | UPC-2 | UPC-3 | |
|----------|------|-------|-------|-------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|---|
| Endogeic | ACCT | | | 1 | 12 | 1 | 18 | 55 | 58 | 29 | 9 | 49 | 36 | 32 | 10 | 43 | |
| | ARR | 27 | 6 | 10 | | | 1 | 2 | 1 | 14 | 28 | 1 | 23 | 6 | 23 | 19 | |
| | KG | | | | | 27 | | | | | | | | | | | |
| | NCCT | | 23 | 8 | 6 | 3 | 10 | 8 | 11 | 30 | 2 | 5 | 1 | 6 | 5 | 24 | |
| | OTL | 2 | | | | 2 | | | | | | | | | | | |
| | OX | | | | | | | 2 | | | 4 | | | | | 10 | |
| | Mt1 | | | | | | | | 11 | | | | | | 19 | | 9 |
| | Mt2 | 2 | | | | 1 | 2 | 1 | | | 1 | | 18 | 7 | 7 | 1 | |
| | Mt3 | | | | | | | | | | 3 | | | | | | |
| Anecic | LT | | 21 | | | | | | | | | | 14 | | | | |
| | LAX | 21 | | 3 | | 2 | | | 1 | | | | | | 19 | | |
| | NCM | | | 3 | | 7 | 8 | 14 | 7 | 13 | 39 | 18 | 6 | 7 | 20 | 3 | |
| | NG | | | | | | 7 | | | 3 | | | 2 | | | | |
| | NLL | | | | | | 33 | | | | | | 2 | | | | |
| | NN | 46 | 40 | 75 | 29 | 39 | 21 | 14 | 11 | 8 | 17 | 10 | 6 | 3 | 1 | 2 | |
| | OcTr | | | | | 10 | | 3 | | | | | | | | 29 | |
| Epigeic | DX | | | | | 2 | | | | | | | | | | | |
| | LC | 2 | 10 | | 53 | 5 | | 1 | | | | | 3 | | | | |
| | Mt4 | | | | | | | | | | 1 | | | | | | |
| | PD | | | | | | | | | | | | | | 1 | | |

Taxa richness (Table 6) varied widely among sites, ranging from 4 to 11 taxa (mean = 7.5). Shannon's diversity index ranged between 0.9 and 1.8 (mean = 1.5), while Pielou's evenness ranged from 0.5 to 0.9 (mean = 0.8).

Table 6. Diversity indices calculated for earthworm communities at the study sites.

| Plot | Taxa richness | Shannon | Evenness |
|-------|---------------|---------|----------|
| PPf-1 | 6 | 1.3 | 0.7 |
| PPf-2 | 5 | 1.4 | 0.9 |
| PPf-3 | 6 | 0.9 | 0.5 |
| PP-1 | 4 | 1.1 | 0.8 |
| PP-2 | 11 | 1.7 | 0.7 |
| PP-3 | 9 | 1.8 | 0.8 |
| UGI-1 | 8 | 1.5 | 0.7 |
| UGI-2 | 6 | 1.4 | 0.8 |
| UGI-3 | 10 | 1.8 | 0.8 |
| UP-1 | 9 | 1.5 | 0.7 |
| UP-2 | 7 | 1.3 | 0.7 |
| UP-3 | 8 | 1.7 | 0.8 |
| UPC-1 | 8 | 1.8 | 0.9 |
| UPC-2 | 9 | 1.7 | 0.8 |
| UPC-3 | 6 | 1.4 | 0.8 |
| Min | 4 | 0.9 | 0.5 |
| Max | 11 | 1.8 | 0.9 |
| Mean | 7 | 1.5 | 0.8 |
| SD | 2 | 0.3 | 0.1 |

3.3.4 Effect of soil and environment variables on earthworms

3.3.4.1. Variable selection procedure results

As indicated by the exploratory one-way ANOVAs performed prior to model fitting, only a few earthworm variables (mainly the relative abundances of endogeic and anecic earthworms) showed interpretable differences across green area categories, whereas no consistent pattern emerged for the others (see Supplementary Materials). Therefore, green area category was not included as an explanatory variable in the linear models. Moreover, including this factor, which comprises multiple levels and a small number of replicates per level, would have reduced model robustness, and its main ecological features are already partly captured by individual predictors describing soil and environmental characteristics.

Separate linear models were fitted for the following response variables: total earthworm abundance and biomass, abundance and biomass by ecological category (anecic, endogeic, epigeic), relative abundance by ecological category, and taxa richness. Abundance and biomass by developmental stage, as well as

Shannon diversity and evenness indices, were not considered as response variables, as their inclusion did not provide additional ecological insight beyond the selected metrics.

Following the variable selection procedure described in Section 3.2.3, the initial model for each biological response included bulk density, clay content, pH, soil organic carbon, Pb content, and patch area as continuous predictors, and vegetation cover (grassland vs. forest) as a categorical predictor.

3.3.4.2 Linear model results

The results of linear models assessing the effects of soil and environmental variables on earthworm abundance, biomass, functional composition, and diversity indices are summarized in Table 7 (a, b). Model for total abundance was not significant ($p > 0.05$), while models for the other earthworm metrics were significant.

Biomass was negatively related to Pb concentration ($p < 0.01$) and showed a weak positive association with SOC. Anecic biomass and abundance both decreased with increasing Pb concentration ($p < 0.05$). As anecic earthworms accounted for about 70% of total biomass, patterns in total biomass largely mirrored those observed for anecics. For endogeic earthworms, abundance and biomass were negatively related to patch area ($p < 0.001$). Endogeic biomass was further negatively related to BD and positively associated with Pb concentration, and slightly higher values were observed in grasslands than in forest sites.

Epigeic abundance increased with bulk density and clay content ($p < 0.01$) and was negatively associated with pH ($p < 0.001$). Biomass was positively related to clay ($p < 0.05$) and patch area ($p < 0.01$) and was higher in grasslands than in forest sites ($p < 0.01$). However, these patterns should be viewed in light of the low representation of epigeic taxa in the samples. The relative abundance of ecological categories showed contrasting responses to Pb concentration, with anecic earthworms decreasing and endogeic increasing as Pb levels rose ($p < 0.01$). Anecic relative abundance was also higher in forest than in grassland sites ($p < 0.01$). Endogeic relative abundance was negatively related to patch area ($p < 0.001$), whereas epigeic relative abundance showed the opposite pattern and was also higher in grasslands ($p < 0.05$). Taxa richness was negatively related to clay content, patch area ($p < 0.05$), and Pb concentration ($p < 0.01$).

Table 7a. Results of linear models for earthworm metrics. Standardized regression coefficients are reported for each predictor. Adjusted R^2 and model p -values are also reported. Veg.cover G: vegetation cover–grassland. Significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; . $p < 0.1$.

| | Tot. ab. | Ane. ab. | End. ab. | Epi. ab. | Tot. bio. | Ane. bio. | End. bio. | Epi. bio. |
|---------------------|-----------|-----------|-------------|------------|-----------|-----------|------------|-----------|
| BD | - | - | - | 11.12 ** | - | - | -12.17 ** | - |
| Clay | - | - | - | 6.9 ** | - | - | - | 1.3 * |
| pH | - | - | - | -11.27 *** | - | - | - | - |
| SOC | - | - | - | - | 53.02 . | - | - | - |
| Pb | - | -112.83 * | - | - | -74.92 ** | -49.9 * | 10.13 *** | - |
| Patch area | -114.62 . | - | -100.25 *** | - | - | - | -17.86 *** | 2.31 ** |
| Veg. cover G | - | - | - | - | - | - | 20.73 * | 6.05 ** |
| <i>Model stats:</i> | | | | | | | | |
| <i>R2 adj</i> | 0.140 | 0.301 | 0.589 | 0.676 | 0.346 | 0.300 | 0.905 | 0.586 |
| <i>p-value</i> | 0.094 | 0.020 | 0.001 | 0.001 | 0.031 | 0.020 | 0.000 | 0.005 |

Table 7b. Results of linear models for earthworm metrics (continued).

| | Ane. rel. ab. | End. rel. ab. | Epi. rel ab. | Taxa rich. |
|---------------------|---------------|---------------|--------------|------------|
| BD | - | - | - | - |
| Clay | - | - | - | -0.9 * |
| pH | - | - | - | - |
| SOC | - | - | - | - |
| Pb | -13.31 ** | 12.74 ** | - | -1.34 ** |
| Patch area | - | -16 *** | 14.19 ** | -0.89 * |
| Veg. cover G | -29.07 ** | - | 24.47 * | - |
| <i>Model stats:</i> | | | | |
| <i>R2 adj</i> | 0.723 | 0.699 | 0.531 | 0.649 |
| <i>p-value</i> | 0.000 | 0.000 | 0.004 | 0.002 |

3.3.5 Main drivers of earthworm community species composition

In the Redundancy Analysis (RDA), bulk density, clay content, pH, soil organic carbon, Pb content, patch area, and vegetation cover were included as explanatory variables, corresponding to the same set of predictors used in the linear models. The RDA revealed a significant relationship between earthworm community species composition and environmental variables ($F = 1.96$, $p = 0.005$). The environmental predictors jointly explained 66.3% of the total constraint variance in species composition, taking into account the adjusted $R^2 = 0.33$. Among canonical axes, only the first (RDA1) was significant ($F = 6.41$, $p = 0.002$), accounting for 46.6% of the constrained variance, while RDA2 explained 17.6% and was not significant ($p = 0.19$) (Fig. 5). Soil pH ($p = 0.001$), bulk density ($p = 0.013$), and organic carbon ($p = 0.017$) were the main variables shaping community composition, whereas vegetation cover showed a marginal effect ($p = 0.086$). Patch area, Pb and clay were not significant.

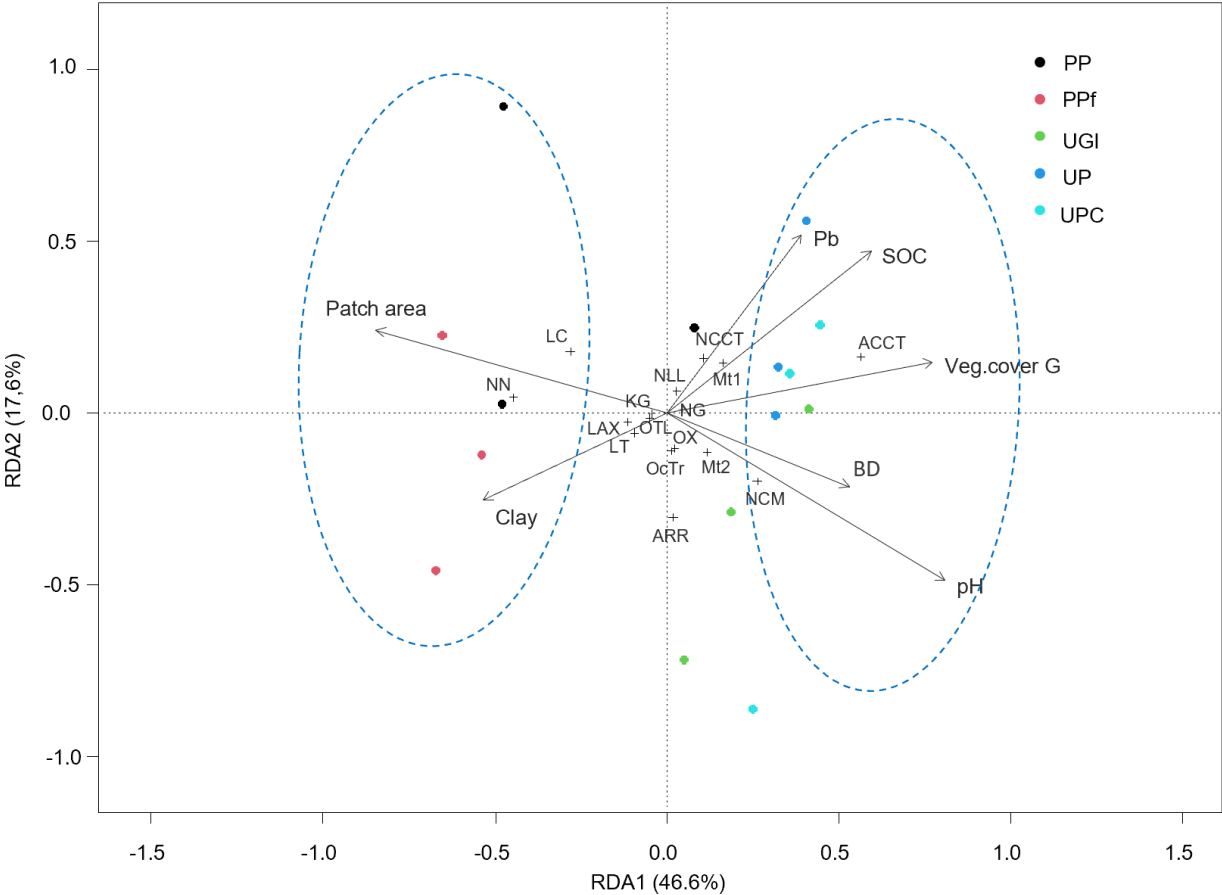


Figure 5. RDA biplot of earthworm species in relation to soil and environmental variables. Crosses represent species, arrows indicate explanatory variables, and points represent sites (coloured by green area category). Circles are shown for visual interpretation only. Veg.cover G: vegetation cover–grassland.

The RDA showed that community structure was mainly shaped by a single environmental gradient, represented by RDA1, and defined primarily by pH, bulk density, SOC and, marginally, by vegetation cover. These variables were oriented in the same direction, opposite to clay content and patch area, which,

although not significant, showed substantial loadings on the same axis. RDA2, although not significant, reflected a secondary pattern in which SOC and Pb varied in the same direction, opposed to pH and BD.

Sites were distributed along RDA1, with peri-urban parks (PP, PPf) located towards the left and urban green areas (UP, UPC, UGI) towards the right of the biplot, following the main soil gradient and partially overlapping, indicating a gradual transition between groups.

The majority of species occupied intermediate positions along RDA1, indicating a weak association with the main soil gradient and suggesting broad ecological tolerance. In contrast, a few taxa tended to be clearly separated from this central grouping. The endogeic *Allolobophora chlorotica* (ACCT) and, to a lesser extent, the anecic *Aporrectodea caliginosa meridionalis* (NCM), were associated with soils with higher pH, BD and SOC, whereas the anecic *Aporrectodea nocturna* (NN) and the epigeic *Lumbricus castaneus* (LC) occurred toward the opposite end. The vertical separation on the right-hand side of the biplot suggested that ACCT was more related to higher SOC and Pb conditions, whereas NCM was more closely associated with higher pH and BD; however, this should be interpreted as a tendency, as RDA2 was not significant. For additional statistical details, see the Supplementary Materials.

3.4 Discussion

This study provides an assessment of earthworm biodiversity in urban environments and examines how earthworm communities are shaped by urbanisation, representing, to our knowledge, the first dataset for Italian cities. We characterised earthworm communities in public green spaces in Milan in terms of abundance, biomass, and functional and taxonomic composition. We also investigated the factors influencing these communities, considering soil properties—including heavy metals—, vegetation cover, land use, and landscape configuration. This study complements the assessments of physical and chemical characteristics of Milan's soils by adding a biological perspective. Interpreting the ecological consequences of urbanisation on soil ecosystems requires approaches that consider soil systems across their physical, chemical, and biological dimensions.

Our previous assessment of chemical and physical properties (Chapter 2) indicated that soils in Milan's green spaces were characterised by low compaction, consistently moderate to high SOC content, and pH values within an ecological favourable range. Urban pressure, however, left a recognisable imprint on several parameters, with bulk density, penetration resistance and pH following a clear urban–peri-urban gradient. Peri-urban areas retained more semi-natural soil features, whereas urban green spaces—particularly central urban parks (UPC) and urban green islands (UGI)—showed stronger signs of alteration, reflecting differences in management intensity, recreational pressure and land-use history. In the subset

of sites analysed for soil fauna, soil properties broadly reflected these patterns, although the gradients were less continuous. A similar spatial trend was observed for heavy metals too, which were not addressed in Chapter 2, as they were collected specifically for soil fauna assessment. Heavy metal concentrations were generally higher in urban parks (UPC and UP) than in peri-urban parks (PP and PPf), consistent with the influence of traffic-related inputs and long-term urban activity. Heavy metals such as Pb, Cu and Zn in urban soils are commonly associated with vehicle traffic, including both historical and ongoing emissions (Manta et al., 2002; Calace et al., 2012), while other sources, such as industrial activities or construction materials, may contribute locally to a lesser extent. Urban green islands, although located in high-traffic areas, showed lower heavy metal levels compared to UPC and UP, likely reflecting their specific management histories (see Chapter 4).

Across the sampling sites, earthworm abundance and biomass varied widely, reflecting the marked heterogeneity in soil conditions, management practices and disturbance regimes that characterises urban ecosystems (Guilland et al., 2018; Xie et al., 2018; Marechal et al., 2021).

Earthworm abundance and biomass (mean \pm SD: 428 ± 256 ind m^{-2} and 128.4 ± 83.0 g m^{-2} , respectively) in Milan's green spaces were high compared with values commonly reported for urban soils, with abundance being particularly elevated. Global syntheses indicate average values of approximately 149 ± 152 ind m^{-2} and 76 ± 82 g m^{-2} in urban environments (Phillips et al., 2025), and studies from Mediterranean temperate urban contexts also report lower abundances (e.g., 163 ind m^{-2} in Montpellier, France; Eydoux et al., 2024). Comparable values have, for example, been reported in particularly favourable urban settings, such as old urban parks (e.g., 437 ind m^{-2} ; Smetak et al., 2007).

A total of 18 earthworm taxa were recorded across the study area, including 13 identified with certainty at the species or subspecies level. At the site scale, taxonomic richness was consistently high (4–11 taxa per site; mean \pm SD: 7.5 ± 2) and exceeded values commonly reported for urban environments in European temperate contexts, where typical values are around 4–5 taxa per site (Amossé et al., 2016; Eydoux et al., 2024).

Taken together, these results indicate that soils in Milan's green spaces support both high earthworm abundance and biomass and high taxonomic richness relative to other urban environments. A more robust ecological interpretation of these values would, however, require comparison with semi-natural reference ecosystems within the same geo-climatic context. At present, however, such reference data are lacking for lowland northern Italy. To our knowledge, the only available Italian study refers to Alpine meadows (Rüdisser et al., 2021), an environment that is not directly comparable to the urban lowland context of Milan and exhibits lower abundance and reduced species richness. Although not directly comparable either, semi-natural grasslands from other temperate European regions provide a broader point of

reference, suggesting that earthworm abundance and species richness in Milan's green spaces are of a similar order of magnitude (Didden, 2001).

Earthworm communities across the study sites were dominated by widespread, generalist species (such as *Allolobophora chlorotica*, *Aporrectodea caliginosa caliginosa* and *A. caliginosa meridionalis*), which are commonly reported as characteristic of urban environments (Eydoux et al., 2024). Alongside these common taxa, a small number of less widespread species with more restricted geographic distributions were also recorded. These included *Octodrilus transpadanus* (Italo-Balkan distribution) and *Koinodrilus georgi* (North-Mediterranean and Central-Eastern Europe), which occurred at few sites but also reached locally high abundances.

Only a single specimen of a non-native species (*Pheretima diffringens*, native to Southeast Asia) was recorded during the survey. This isolated occurrence is consistent with previous evidence indicating that invasive earthworms are currently not a major issue in European urban environments, where non-native taxa are reported sporadically and mostly in local, non-quantitative studies (Rota, 2013; Phillips et al., 2025).

In terms of ecological categories, when considering all sites combined, sampled earthworms were characterised by a near-balanced contribution of endogeics and anecics, whereas epigeic earthworms were rare, accounting for only about 2% of individuals overall (mean abundance across sites: 8.3 ± 11.7 ind m^{-2}). A reduced contribution of epigeic taxa is commonly reported across urban, agricultural, and natural soils (Burton et al., 2024; Phillips et al., 2025); however, the very low values observed here remain uncommon. Given their low abundance and strongly uneven distribution across sites, relationships involving epigeic earthworms are expected to be more sensitive to local conditions and sampling effects, and model-based results for this group should therefore be interpreted with caution.

Patterns emerging from the linear models indicate that earthworm communities in Milan's green spaces respond to a combination of soil and environmental factors. Among the soil variables considered, Pb concentration showed the most consistent associations with community attributes, whereas bulk density, soil organic carbon and clay content showed only limited effects.

The negative response of anecic earthworms to increasing Pb levels indicates that this ecological group is particularly sensitive to heavy metal contamination in urban soils. Because anecics account for a large share of total biomass, their decline contributes to the overall reduction in earthworm biomass. Pb concentration was also associated with a shift in functional composition: the relative contribution of anecic earthworms decreased with increasing Pb levels, while endogeic earthworms became proportionally more dominant, suggesting that endogeics were less affected by higher metal concentrations, possibly reflecting differences in habitat use and feeding behaviour that reduce their exposure to contamination. In parallel,

the positive association between Pb and endogeic absolute biomass may reflect reduced competition with anecic earthworms. Moreover, Pb also affected negatively taxonomic richness.

These responses are consistent with the relatively limited available evidence on the effects of heavy metals on earthworm communities in urban soils, which points to negative impacts on abundance, biomass and species richness (Pižl & Jones, 1995a). For example, in a study on urban soils in Paris, Chatelain et al. (2024) reported negative correlations between Cu, Hg, Zn, and Pb concentrations and total earthworm abundance, identifying heavy metals as key factors influencing earthworm communities at the local scale. In our work, Pb was retained as a predictor because it occurred at the highest concentrations in the study area and is among the heavy metals most frequently reported to affect earthworm communities (Pižl & Jones, 1995a). However, Pb concentrations were strongly correlated with other traffic-related metals, such as Zn and Cu, which are also known to affect soil biota (Sivakumar, 2015). As a result, the observed patterns cannot be attributed to Pb alone, but likely reflect the combined or interacting effects of multiple metals (Chatelain et al., 2024). In this context, Pb can be interpreted as a proxy for overall heavy metal pressure in Milan's urban soils.

Bulk density (measured range: 0.85–1.27 g cm⁻³) exerted only a limited influence on earthworm communities, showing a moderate negative effect on endogeic biomass but no consistent relationships with other earthworm community attributes. This weak response is consistent with the generally favourable physical status of the studied soils, which were unlikely to impose strong constraints on earthworm activity. Even in sites characterised by relatively higher bulk density—primarily historical parks in the city centre subject to long-term intensive management and trampling—values remained below thresholds commonly associated with critical soil compaction (Beylich et al., 2010; USDA, 2023). In contrast, strong negative effects of soil compaction on urban earthworm communities have been reported at substantially higher bulk density levels (e.g. 1.3–1.6 g cm⁻³; Smetak et al., 2007).

Clay content showed only weak associations with earthworm communities and was negatively related to taxonomic richness, in contrast with patterns reported in the literature (Phillips et al., 2025). In the present study, however, clay content varied within a relatively narrow range and soil texture was largely uniform (loam to sandy loam), conditions that likely limited the emergence of clear and ecologically meaningful effects on earthworm community attributes.

Soil pH did not show consistent relationships with earthworm community attributes. The observed pH range (4.7–7.5) overlapped with values reported as suitable for most temperate earthworm species (5–7.4; Curry, 2004), which likely explains the absence of clear pH-driven patterns. Similar weak pH effects have been reported in urban studies with comparable pH ranges (Eydoux et al., 2024), whereas stronger effects tend to emerge only along broader pH gradients (Xie et al., 2018). Notably, pH showed a negative

relationship with epigeic abundance; however, this result should be interpreted with caution given the very low and uneven representation of epigeic earthworms across sites.

Similarly, despite being widely recognised as a key driver of earthworm abundance and biomass (Phillips et al., 2025), soil organic carbon exerted only a limited influence in this study, showing a weak positive association with total biomass. SOC values were consistently moderate to high across sites, suggesting that organic matter availability was unlikely to represent a limiting factor for earthworm communities.

Beyond Pb concentration, patch area, defined as the surface of the continuous green space surrounding each sampling plot, emerged as the other factor most strongly shaping earthworm communities, particularly their ecological structuring. This is consistent with the central role of landscape attributes reported for urban earthworm assemblages (Xie et al., 2018; Eydoux et al., 2024). Increasing patch area was associated with lower abundance and biomass of endogeic earthworms, together with a decline in their relative abundance. In addition, taxonomic richness increased with decreasing patch area.

These results are surprising and difficult to explain, as we can assume that the increase of patch area should provide better soil habitat for earthworms. In fact, patch area was included to reflect potential ecological connectivity for earthworm communities within the urban matrix. However, the observed patterns suggest that patch area may primarily act as a proxy for the broader urban-peri-urban pressure gradient observed in the city, rather than as a direct measure of connectivity effects. In Milan, in fact, smaller green patches are concentrated in the city centre, whereas patch size increases progressively towards peri-urban areas.

Vegetation cover also influenced earthworm functional composition, in line with previous findings from urban soils (Phillips et al., 2025). Anecic earthworms showed a higher relative abundance in forested sites compared to grasslands, whereas endogeic biomass was higher in grassland sites. Forest vegetation likely favours anecic earthworms, which are generally more ecologically demanding, through the presence of an undisturbed litter layer that provides food, refuge, and buffered microclimatic conditions (Lee, 1985).

The patterns emerging from the models could help explain the differences in earthworm functional composition observed across green area categories. Clear contrasts were observed between urban and peri-urban green spaces, indicating that different levels of urban pressure shape the relative abundance of ecological categories. Endogeic earthworms prevailed in urban parks (UPC and UP) and in urban green islands (with one exception), whereas anecic earthworms predominated in peri-urban parks, particularly in forested areas (PPf). Epigeic earthworms, although rare overall, occurred in higher proportions in peri-urban parks.

Urban green spaces, particularly UPC and UGI, are characterised by higher levels of anthropogenic pressure. They are subject to intensive management and frequent trampling, resulting in stronger surface disturbance. In contrast, peri-urban parks experience lower management intensity and reduced human use and thus exhibit environmental conditions closer to semi-natural systems. Surface disturbance associated with intensive management and repeated trampling is known to negatively affect earthworm communities (Pižl and Schlaghamersky, 2007; Smetak et al., 2007), particularly surface-feeding and surface-dwelling species such as anecic and epigeic earthworms (Marechal et al., 2024). Accordingly, being more tolerant to disturbance, endogeic earthworms dominated green spaces in urban context, whereas peri-urban sites supported higher proportions of anecic and, to a lesser extent, epigeic earthworms. Differences in heavy metal levels, identified as key drivers in this study, further contribute to these patterns.

These results are consistent with previous findings (Li et al., 2020). In some highly disturbed contexts, earthworm communities are strongly dominated by endogeic species, in some cases accounting for up to 90% of total abundance (Maréchal et al., 2021), as occasionally observed in our study. For the proper functioning of soils, however, the balanced representation of all three ecological categories is essential; for example, epigeic earthworms play a key role in the decomposition of SOM, whereas anecic are particularly important for water infiltration (Brown et al., 2000; Shipitalo and Le Bayon, 2004).

Redundancy analysis showed that earthworm species composition across Milan's green spaces was structured along a main environmental gradient defined primarily by soil pH, bulk density and soil organic carbon, with vegetation cover playing a marginal role. The main RDA axis broadly mirrored the contrast between peri-urban and urban green spaces, consistent with the spatial patterns previously observed for soil properties and indicative of an underlying gradient of urban pressure. Most taxa occupied intermediate positions along the gradient indicating broad ecological tolerance and weak association with the main soil gradient, whereas a few species clearly separated from the central grouping. In particular, the widespread endogeic species *Allolobophora chlorotica* and the anecic *A. caliginosa meridionalis* were associated with soils with higher pH, bulk density and organic carbon, conditions more frequently found in urban green spaces subject to intensive management and human use. Moreover, *A. chlorotica* seems to be associated with higher Pb concentrations, suggesting also that this species may be less affected by heavy metal contamination. At the opposite end of the gradient, the epigeic *Lumbricus castaneus* and the anecic *Aporrectodea nocturna* were associated with soils less altered by urban pressures, typical of peri-urban context.

One limitation of this study that should be considered is the relatively small sample size (n = 15) used for modelling earthworm responses in relation to multiple predictors. Although predictors were selected

based on ecological relevance and model selection was performed using AICc to account for small sample size, the limited number of observations may still affect the stability of the models. Nevertheless, this approach allows the identification of general ecological patterns. Increasing sample size in future studies would help to further support these findings.

3.5 Conclusions

This work contributes to the assessment of urban earthworm biodiversity and its environmental drivers and represents the first study explicitly addressing earthworm communities in Italian urban environments. Earthworm abundance, biomass, and taxonomic richness observed in Milan's green spaces were consistently high, indicating that urban soils can sustain diverse and abundant earthworm communities under favourable conditions. Given the key role of earthworms in soil functioning, including soil structure modification, water regulation, organic matter dynamics and nutrient cycling, these findings suggest that Milan's green spaces—from urban to peri-urban—are generally able to support good soil quality.

Among the environmental and soil factors examined heavy metals—reflected by Pb concentration—and patch area emerged as the most relevant drivers structuring earthworm communities. Increasing Pb levels, associated with long-term urban inputs, influenced earthworms at multiple levels, reducing biomass and taxonomic richness and altering functional composition. Anecic earthworms were particularly sensitive to heavy metals, whereas endogeics were less affected. Patch area, rather than reflecting ecological connectivity alone, appeared to capture a broader urban–peri-urban gradient of human pressures, separating long-term, intensively used central green spaces from larger peri-urban parks with more semi-natural characteristics.

Urban pressures related to management intensity, human use and associated soil alterations affected earthworm ecological groups unevenly, resulting in clear differences in functional composition between urban and peri-urban green spaces. Endogeic species, more tolerant to disturbance, predominated in urban contexts, whereas peri-urban parks—including forested areas—supported higher proportions of anecic and epigeic earthworms.

As earthworms are key elements of soil ecosystems, understanding how urban pressures shape their diversity and functional composition is essential to evaluate the capacity of urban soils to sustain ecosystem functions and to inform sustainable green space management.

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Supplementary Materials

Table S1. Heavy metals concentration across the sampling sites. Values refer to the 0–20 cm layer.

| Plot | Pb | Cu | Ni | Cr | Zn |
|-------------|-----------|-----------|-----------|-----------|-----------|
| PPf-1 | 106 | 25 | 38 | 65 | 59 |
| PPf-2 | 167 | 37 | 37 | 71 | 59 |
| PPf-3 | 86 | 23 | 32 | 59 | 63 |
| PP-1 | 177 | 35 | 39 | 70 | 69 |
| PP-2 | 54 | 15 | 28 | 57 | 43 |
| PP-3 | 77 | 41 | 26 | 49 | 54 |
| UP-1 | 66 | 26 | 48 | 61 | 63 |
| UP-2 | 270 | 191 | 57 | 74 | 178 |
| UP-3 | 176 | 108 | 69 | 117 | 125 |
| UGI-1 | 55 | 35 | 55 | 73 | 61 |
| UGI-2 | 125 | 62 | 48 | 63 | 88 |
| UGI-3 | 88 | 39 | 47 | 60 | 78 |
| UPC-1 | 246 | 93 | 48 | 83 | 125 |
| UPC-2 | 126 | 58 | 44 | 73 | 71 |
| UPC-3 | 334 | 147 | 52 | 75 | 164 |

Table S2. Earthworm abundance and biomass across the study sites, reported as total values and by developmental stage.

| Plot | Earthworm abundance (ind m ⁻²) | | | | Earthworm biomass (g m ⁻²) | | | |
|-------|--|-----------|-----------|--------|--|-----------|-----------|--------|
| | Total | Juveniles | Subadults | Adults | Total | Juveniles | Subadults | Adults |
| PPf-1 | 192 | 144 | 24 | 24 | 55.9 | 35.0 | 17.6 | 3.3 |
| PPf-2 | 192 | 156 | 16 | 20 | 74.5 | 30.5 | 11.4 | 32.6 |
| PPf-3 | 508 | 352 | 84 | 72 | 231.3 | 92.0 | 51.4 | 87.9 |
| PP-1 | 68 | 12 | 16 | 40 | 26.1 | 2.3 | 1.8 | 22.0 |
| PP-2 | 396 | 208 | 36 | 152 | 159.2 | 63.7 | 19.8 | 75.6 |
| PP-3 | 840 | 588 | 52 | 200 | 332.8 | 114.7 | 32.2 | 185.9 |
| UP-1 | 352 | 124 | 68 | 160 | 108.1 | 15.7 | 21.4 | 71.0 |
| UP-2 | 396 | 128 | 32 | 236 | 119.5 | 26.5 | 8.3 | 84.6 |
| UP-3 | 316 | 68 | 60 | 188 | 120.5 | 6.2 | 16.6 | 97.8 |
| UGI-1 | 1104 | 840 | 80 | 184 | 253.4 | 113.8 | 34.4 | 105.2 |
| UGI-2 | 320 | 128 | 24 | 168 | 79.0 | 18.3 | 4.6 | 56.2 |
| UGI-3 | 448 | 232 | 32 | 184 | 104.0 | 26.1 | 3.9 | 74.1 |
| UPC-1 | 380 | 276 | 12 | 92 | 80.3 | 47.5 | 5.3 | 27.4 |
| UPC-2 | 504 | 360 | 44 | 100 | 99.0 | 29.0 | 11.4 | 58.5 |
| UPC-3 | 400 | 176 | 24 | 200 | 81.9 | 27.5 | 2.1 | 52.3 |

Table S3. Mean (\pm SD) values of earthworm abundance and biomass (total and by ecological category) for each green area category.

| | | Earthworm abundance (ind m ⁻²) | | | | Earthworm biomass (g m ⁻²) | | | |
|------------|------|--|--------|----------|---------|--|--------|----------|---------|
| | | Total | Anecic | Endogeic | Epigeic | Total | Anecic | Endogeic | Epigeic |
| PPf | Mean | 297 | 219 | 71 | 8 | 120.6 | 109.6 | 10.8 | 0.3 |
| | SD | 182 | 168 | 22 | 11 | 96.3 | 89.5 | 7.2 | 0.4 |
| PP | Mean | 435 | 276 | 136 | 23 | 172.7 | 149.8 | 20.0 | 4.2 |
| | SD | 387 | 281 | 124 | 17 | 153.8 | 142.2 | 15.1 | 6.0 |
| UP | Mean | 355 | 85 | 268 | 1 | 116.0 | 54.5 | 61.5 | 0.0 |
| | SD | 40 | 20 | 45 | 2 | 6.9 | 7.1 | 14.0 | 0.1 |
| UGI | Mean | 624 | 281 | 333 | 9 | 145.5 | 105.9 | 38.3 | 1.3 |
| | SD | 421 | 294 | 121 | 8 | 94.3 | 92.7 | 2.6 | 1.5 |
| UPC | Mean | 428 | 128 | 299 | 0 | 87.1 | 38.2 | 48.2 | 0.0 |
| | SD | 67 | 117 | 71 | 0 | 10.4 | 27.0 | 18.8 | 0.0 |

Table S4. Abundance of earthworm taxa (ind m²) across the study sites. Taxon codes are reported in Table 4. LAX and OX refer to genus-level identifications of *Lumbricus (anecic)* and *Octolasion*, respectively, used when identification to species level was not possible.

| | | PPf-1 | PPf-2 | PPf-3 | PP-1 | PP-2 | PP-3 | UP-1 | UP-2 | UP-3 | UGI-1 | UGI-2 | UGI-3 | UPC-1 | UPC-2 | UPC-3 |
|-----------------|------|-------|-------|-------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|
| <i>Endogeic</i> | ACCT | 0 | 0 | 4 | 8 | 4 | 148 | 192 | 228 | 92 | 100 | 156 | 160 | 120 | 52 | 172 |
| | ARR | 52 | 12 | 52 | 0 | 0 | 12 | 8 | 4 | 44 | 308 | 4 | 104 | 24 | 116 | 76 |
| | KG | 0 | 0 | 0 | 0 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | NCCT | 0 | 44 | 40 | 4 | 12 | 80 | 28 | 44 | 96 | 20 | 16 | 4 | 24 | 24 | 96 |
| | OTL | 4 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | OX | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 40 | 0 | 0 | 0 | 52 | 0 |
| | Mt1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 72 | 0 | 36 |
| | Mt2 | 4 | 0 | 0 | 0 | 4 | 20 | 4 | 0 | 4 | 0 | 56 | 32 | 28 | 4 | 0 |
| | Mt3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Anecic</i> | LT | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 64 | 0 | 0 | 0 |
| | LAX | 40 | 0 | 16 | 0 | 8 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 72 | 0 | 0 |
| | NCM | 0 | 0 | 16 | 0 | 28 | 68 | 48 | 28 | 40 | 436 | 56 | 28 | 28 | 100 | 12 |
| | NG | 0 | 0 | 0 | 0 | 0 | 56 | 0 | 0 | 8 | 0 | 0 | 8 | 0 | 0 | 0 |
| | NLL | 0 | 0 | 0 | 0 | 0 | 276 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 |
| | NN | 88 | 76 | 380 | 20 | 156 | 176 | 48 | 44 | 24 | 184 | 32 | 28 | 12 | 4 | 8 |
| | OcTr | 0 | 0 | 0 | 0 | 40 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 148 | 0 |
| <i>Epigeic</i> | DX | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | LC | 4 | 20 | 0 | 36 | 20 | 0 | 4 | 0 | 0 | 4 | 0 | 12 | 0 | 0 | 0 |
| | Mt4 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 |
| | PD | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |

Table S5. Biomass of earthworm taxa (g m⁻²) across the study sites. Taxon codes are reported in Table 4. LAX and OX refer to genus-level identifications of *Lumbricus* (anecic) and *Octolasion*, respectively, used when identification to species level was not possible.

| | | PPf-1 | PPf-2 | PPf-3 | PP-1 | PP-2 | PP-3 | UP-1 | UP-2 | UP-3 | UGI-1 | UGI-2 | UGI-3 | UPC-1 | UPC-2 | UPC-3 |
|-----------------|------|-------|-------|-------|------|------|-------|------|------|------|-------|-------|-------|-------|-------|-------|
| <i>Endogeic</i> | ACCT | 0 | 0 | 1.0 | 2.1 | 0.5 | 20.8 | 40.7 | 49.2 | 34.2 | 11.2 | 32.4 | 26.7 | 18.6 | 5.7 | 34.7 |
| | ARR | 3.7 | 1.3 | 9.6 | 0 | 0 | 0.9 | 1.8 | 0.7 | 11.3 | 26.1 | 0.5 | 6.6 | 2.7 | 18.2 | 9.1 |
| | KG | 0 | 0 | 0 | 0 | 14.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | NCCT | 0 | 7.5 | 8.1 | 1.2 | 4.5 | 8.9 | 2.3 | 4.9 | 22.9 | 0.8 | 3.0 | 0.6 | 4.9 | 2.0 | 17.2 |
| | OTL | 1.0 | 0 | 0 | 0 | 3.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | OX | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 | 0 | 0 | 0.8 | 0 | 0 | 0 | 2.3 | 0 |
| | Mt1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15.4 | 0 | 0 | 0 | 0 | 18.6 | 0 | 5.6 |
| | Mt2 | 0.1 | 0 | 0 | 0 | 1.2 | 1.9 | 0.3 | 0 | 0.6 | 0 | 4.6 | 1.5 | 4.2 | 0.8 | 0 |
| | Mt3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Anecic</i> | LT | 0 | 14.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19.0 | 0 | 0 | 0 |
| | LAX | 8.1 | 0 | 2.6 | 0 | 1.1 | 0 | 0 | 4.3 | 0 | 0 | 0 | 0 | 10.5 | 0 | 0 |
| | NCM | 0 | 0 | 5.3 | 0 | 11.3 | 28.1 | 23.5 | 7.7 | 20.4 | 127.3 | 14.9 | 11.9 | 9.8 | 31.2 | 5.9 |
| | NG | 0 | 0 | 0 | 0 | 0 | 33.5 | 0 | 0 | 8.2 | 0 | 0 | 3.6 | 0 | 0 | 0 |
| | NLL | 0 | 0 | 0 | 0 | 0 | 129.3 | 0 | 0 | 0 | 0 | 0 | 6.5 | 0 | 0 | 0 |
| | NN | 43.0 | 50.5 | 204.7 | 16.4 | 97.2 | 108.5 | 27.8 | 37.3 | 23.0 | 84.3 | 23.6 | 26.7 | 11.1 | 3.5 | 9.4 |
| | OcTr | 0 | 0 | 0 | 0 | 23.8 | 0 | 11.3 | 0 | 0 | 0 | 0 | 0 | 0 | 33.1 | 0 |
| <i>Epigeic</i> | DX | 0 | 0 | 0 | 0 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | LC | 0.1 | 0.7 | 0.0 | 6.4 | 1.0 | 0.0 | 0.1 | 0 | 0 | 1.1 | 0 | 0.9 | 0 | 0 | 0 |
| | Mt4 | 0 | 0 | 0 | 0 | 0 | 0.8 | 0 | 0 | 0 | 1.9 | 0 | 0 | 0 | 0 | 0 |
| | PD | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.1 | 0 | |

ANOVA AND POST HOC RESULTS FOR EARTHWORM METRICS ACROSS GREEN AREA CATEGORIES

Summary of one-way ANOVA results testing the effect of green area categories (*green_area*) on earthworm abundance, biomass and relative abundance. Only significant ANOVA are reported.

For each model, the ANOVA F-test statistics (degrees of freedom, p-value, R^2 and adjusted R^2) are reported, followed by Tukey post hoc comparisons based on estimated marginal means (emmeans). Different groups indicate statistically significant differences among green area types ($\alpha = 0.05$).

Abbreviations: PPf = peri-urban forest, PP = peri-urban park, UP = urban park, UGI = urban green island, UPC = urban park centre.

Endogeic abundance:

ANOVA: $F(4,10) = 5.04$, $p = 0.017$, $R^2 = 0.67$ (adj. 0.54)

Tukey (emmeans): PPf (70.7) < PP (136.0) = UP (268.0) = UPC (298.7) < UGI (333.3)

Endogeic biomass:

ANOVA: $F(4,10) = 7.65$, $p = 0.004$, $R^2 = 0.75$ (adj. 0.66)

Tukey (emmeans): PPf (10.7) < PP (20.0) = UGI (38.3) < UPC (48.2) < UP (61.5)

Endogeic relative abundance:

ANOVA: $F(4,10) = 8.93$, $p = 0.002$, $R^2 = 0.78$ (adj. 0.69)

Tukey (emmeans): PPf (26.4) = PP (27.6) < UGI (60.6) < UPC (71.6) = UP (75.4)

Anecic relative abundance:

ANOVA: $F(4,10) = 3.92$, $p = 0.036$, $R^2 = 0.61$ (adj. 0.45)

Tukey (emmeans): UP (24.2) < UPC (28.2) = UGI (38.0) = PP (52.2) < PPf (69.4)

REDUNDANCY ANALYSIS (RDA) SCORES FOR VARIABLES, SPECIES, AND SITES

Abbreviations: SOC = soil organic carbon, BD = bulk density, PR = penetration resistance; Veg. cover G: vegetation cover–grassland. PPF: peri-urban forest, PP: peri-urban park, UP: urban park, UGI: urban green island, UPC: urban park centre. Species codes are reported in Table 4.

Species scores

| | RDA1 | RDA2 |
|------|-------------|--------------|
| ACCT | 0.56562409 | 0.162986153 |
| ARR | 0.01809347 | -0.303577124 |
| KG | -0.04349818 | -0.006074159 |
| NCCT | 0.10536604 | 0.159677146 |
| OTL | -0.05153406 | -0.015582556 |
| LT | -0.09405211 | -0.058456794 |
| NCM | 0.26243477 | -0.199469400 |
| OX | 0.02489165 | -0.104547318 |
| LAX | -0.11240929 | -0.028369629 |
| NG | 0.04612939 | 0.010408535 |
| NLL | 0.02750137 | 0.061373714 |
| NN | -0.44517666 | 0.044259497 |
| Octr | 0.01206161 | -0.111634778 |
| LC | -0.28151529 | 0.176143655 |
| Mt1 | 0.16568565 | 0.142127848 |
| Mt2 | 0.11702366 | -0.114545071 |

Sites scores

| | RDA1 | RDA2 |
|-------|-------------|-------------|
| UPC-1 | 0.35582652 | 0.11522635 |
| UPC-2 | 0.25007604 | -0.86388142 |
| PP-1 | -0.47606809 | 0.89239680 |
| PPf-1 | -0.67347504 | -0.45871603 |
| PPf-2 | -0.65269982 | 0.22508538 |
| PP-2 | -0.48110313 | 0.02497313 |
| UP-1 | 0.32267279 | 0.13277586 |
| PP-3 | 0.07955451 | 0.24884169 |
| PPf-3 | -0.53773300 | -0.12435885 |
| UP-2 | 0.40323779 | 0.55854799 |
| UP-3 | 0.31562527 | -0.00882416 |
| UGI-1 | 0.04838627 | -0.71815888 |
| UGI-2 | 0.41261630 | 0.01109790 |
| UPC-3 | 0.44487122 | 0.25544960 |
| UGI-3 | 0.18821236 | -0.29045536 |

Environmental and soil variables scores

| | RDA1 | RDA2 |
|------------|------------|------------|
| BD | 0.5301295 | -0.2170572 |
| pH | 0.8082083 | -0.4894502 |
| SOC | 0.5934034 | 0.4687614 |
| Clay | -0.5353816 | -0.2552619 |
| Patch_area | -0.8444335 | 0.2414277 |
| Pb | 0.3914936 | 0.5187461 |
| Veg_coverG | 0.7729322 | 0.1484524 |

CHAPTER 4

SOIL BIOLOGICAL QUALITY INDEX BASED ON MICROARTHROPODS (QBS-ar)

Abstract

Urban green spaces can play a crucial role in sustaining soil ecosystem functions and biodiversity, yet the ecological condition of urban soils remains poorly understood. To help address this gap, the QBS-ar index (Soil Biological Quality based on microarthropods) was applied to assess soil microarthropods communities and evaluate the biological quality of soils in public green spaces in Milan. The influence of soil properties, land use, management intensity and site-specific histories on QBS-ar values was evaluated. The study was carried out in a subset of 15 sites selected from those investigated in Chapter 2.

QBS-ar values ranged from 61 to 136 (mean \pm SD: 88 ± 22), indicating a general moderate biological quality of Milan's soils, in line with values reported for other Italian cities. Overall, QBS-ar showed limited alignment with the main urban–peri-urban gradient identified for soil physical and chemical properties. The index was negatively associated with bulk density and penetration resistance and weakly positive related to the C:N ratio, confirming its sensitivity to soil compaction and to litter quantity and quality. Significant differences emerged among green area categories: peri-urban forests displayed the highest QBS-ar values (mean \pm SD: 118 ± 21), confirming their role as key habitats for soil microarthropods, whereas grasslands in central urban parks showed the lowest values (mean \pm SD: 69 ± 9), consistent with higher disturbance levels. In contrast, peri-urban grasslands and small urban green islands exhibited less straightforward patterns, as QBS-ar values were not fully explained by current soil conditions or management intensity, pointing to the influence of site-specific events capable of overriding general urban pressure gradients.

Overall, QBS-ar did not provide an integrated representation of the environmental drivers and urban pressures shaping soils across the city, and its use as a general indicator of soil status therefore requires caution. The index was only weakly responsive to fine-scale differences in management, disturbance intensity or soil properties. Nevertheless, QBS-ar proved to be a simple and synthetic tool to characterise soil microarthropod communities in urban green spaces. This study provides a first baseline for microarthropod-based assessments in Milan's urban soils and supports the integration of biological indicators with physical and chemical properties to better capture the complexity of urban soil systems.

4.1 Introduction

Soils in urban green spaces play a central role in urban sustainability, supporting key ecosystem functions and services (O’Riordan et al., 2021). Growing evidence shows that urban soils are not uniformly degraded, and that they often retain physical, chemical and biological properties comparable to those of semi-natural soils despite urbanisation pressures, also acting as reservoirs of biodiversity (Guilland et al., 2018; Pouyat et al., 2020). Yet, their ecological value is still not fully acknowledged by city managers and urban planners (Blanchart et al., 2018). There is therefore a need to improve the understanding of the role and condition of urban soils. To this end, tools that provide an overall assessment of soil quality— and track its changes over time to prevent degradation or evaluate management and restoration outcomes—are essential (Pavao-Zuckermann, 2012).

Currently, the assessment and monitoring of urban soil conditions still rely predominantly on individual parameters—mainly chemical or physical—which, while useful for addressing specific issues or targeted interventions, offer only a partial view of soil functioning as an ecosystem (Joimel et al., 2017). In this perspective, biological indicators provide a valuable complement to traditional physical and chemical measurements: they integrate the cumulative influence of environmental conditions, respond sensitively to ecosystem dynamics and offer synthetic, easily interpretable information. Several biological indices based on soil fauna have been developed (Menta, 2012; Paz-Ferreiro and Fu, 2016), assessing both soil ecological conditions and the status of belowground faunal communities. Many of them rely on soil microarthropods, widely recognised as effective indicators of soil conditions and ecological change (Van Straalen, 1998; Menta et al., 2018b).

Microarthropods are one of the most abundant and diverse groups of soil fauna and occupy multiple trophic levels – from detritivores to herbivores, predators and parasites – giving them a central role in the soil food web. Because most species are detritivores, they are key elements of the detritus-based trophic web and contribute significantly to organic matter decomposition and nutrient cycling (Lavelle et al., 2006; Bardgett and van der Putten, 2014). Microarthropods possess several traits that make them particularly suitable as bioindicators. Their limited mobility and central role in the soil food web make them highly sensitive to micro-environmental changes (Paoletti, 1999; McIntyre et al., 2001). They are also abundant, react rapidly to soil disturbance and are easy to sample (Van Straalen, 1998; McIntyre et al., 2001).

Sensitivity to soil disturbance varies greatly across microarthropod taxa. Soil-specialist groups—such as Protura, Pauropoda and euedaphic Collembola—are particularly vulnerable to environmental change (Menta, 2012), which often shifts communities toward dominance by a few tolerant taxa, while more sensitive groups decline (Sattler et al., 2010).

For this reason, total abundance or taxonomic richness alone do not necessarily reflect soil quality (Menta and Remelli, 2020), as increases in tolerant taxa can mask the decline of more sensitive groups (Horváth et al., 2021; Rodríguez-Pajares et al., 2025). In contrast, the presence or absence of soil-specialist taxa can provide ecologically meaningful information, even at coarse taxonomic resolution (Santorufu et al., 2012; Maisto et al., 2017).

The QBS-ar (Soil Biological Quality index based on microarthropods), developed in Italy by Parisi et al. (2001, 2005), was specifically designed to assess soil quality by focusing on the presence of soil-specialist microarthropods. The index integrates two key aspects: the diversity of microarthropod groups and their degree of adaptation to soil life, which reflects their vulnerability to disturbance (Parisi et al., 2005; Menta et al., 2018a). The method relies on easily recognisable morphological traits indicative of edaphic specialisation and does not require species-level identification or counting individuals, as it is based on higher taxonomic groups. These characteristics make the QBS-ar rapid, inexpensive, and suitable for large-scale assessments, even by users with limited taxonomic expertise (Fusco et al., 2023; Naglič et al., 2025). Thanks to its simplicity, the QBS-ar has been adopted for monitoring purposes by Italian regional environmental agencies (ARPA) and has been proposed as a standard protocol in European monitoring frameworks (Firbank et al., 2017). To date, the majority of QBS-ar applications have been carried out in Italy, although studies from other countries—mainly within Europe—are increasingly available (Menta et al., 2018a).

Most QBS-ar studies have focused on agricultural (Gallese et al., 2025; Naglič et al., 2025), forest (Blasi et al., 2013) and grassland soils (Menta et al., 2011), including alpine environments (Fusco et al., 2023), whereas urban areas have received comparatively little attention (Gardini et al., 2025). This limited application reflects the broader scarcity of research on soil microarthropod communities in cities (Huang et al., 2020) and, more generally on the biological quality of urban soils (Tóth et al., 2023). Available studies in urban soils show that QBS-ar values can vary widely even within the same city, ranging from low to high scores (Santorufu et al., 2012; Horváth et al., 2021), reflecting the heterogeneity of land uses, soil properties and disturbance regimes typical of urban environments. Despite this variability, the QBS-ar often indicate medium-to-high biological quality in urban soils (Magro et al., 2013; Tóth et al., 2023), in some cases reaching levels comparable to those of natural environments (Ungaro et al., 2022; Gardini et al., 2025).

The QBS-ar index responds to the ecological factors that shape the occurrence of soil-specialist microarthropods and, consequently, the composition and functional structure of belowground communities. It discriminates effectively between contrasting land uses and to detect the effects of management practices—particularly in agricultural systems—although its sensitivity to subtle

management variations has been questioned (Naglič et al., 2025). The index is also highly responsive to soil compaction (Menta et al., 2012), an important driver of changes in soil-dwelling microarthropod communities (Devigne et al., 2016).

In urban soils, QBS-ar responds to environmental factors that reflect both soil conditions and site-level management. Several studies report negative associations with heavy metal content (Santorufó et al., 2012; Horváth et al., 2021) and soil compaction (Ungaro et al., 2022; Gardini et al., 2025), two key forms of disturbance in urban environments. QBS-ar also depends on vegetation cover and litter presence or disturbance (Ungaro et al., 2022; Gardini et al., 2025; Rodríguez-Pajares et al., 2025), which shape the availability of suitable microhabitats for soil fauna. In addition, positive associations with soil organic matter and soil C:N ratio (Magro et al., 2013; Ungaro et al., 2022; Tóth et al., 2023) reflect the influence of resource availability on edaphic microarthropods. Urban land-use differences, which often reflect contrasting levels of management and anthropogenic pressure, are also captured by QBS-ar, with lower values typically occurring in more intensively managed or frequently disturbed sites (Maisto et al., 2017; Gardini et al., 2025).

This study extends the integrated investigation of Milan's urban soils initiated in the previous chapters, which examined their physical and chemical properties and earthworm communities. Here, we focus on soil microarthropods, using the QBS-ar index to provide the first systematic assessment of biological soil quality across the city's public green spaces.

We considered a range of green-area categories, including urban and peri-urban parks (both grassland and forested areas) and small urban green islands, selected to capture contrasting conditions in vegetation structure, management intensity, land-use history and urban context. Our aim was to evaluate how microarthropod communities, as reflected by QBS-ar values, vary across green areas and to identify the environmental drivers—including soil properties, management intensity and site context—that may contribute to shaping their patterns, as well as to assess the degree to which the index captures the effects of urban pressures on soils. In doing so, the study advances a more integrated understanding of urban soils and provides a critical evaluation of the usefulness and limitations of QBS-ar as a tool for assessing and monitoring biological soil quality in heterogeneous and complex urban ecosystems.

4.2 Materials and methods

4.2.1 Study area and sampling design

The research focused on public green spaces across the city of Milan. Microarthropod sampling was conducted during the same field campaign and in the same sites used for the earthworm survey (Chapter 3). These sites were selected from those investigated in Chapter 2, which analyzed the chemical and physical properties of Milan's soils and provided the background for the present study. A subset of 15 sites was used for soil fauna sampling (Fig. 1).

Sites were chosen to represent the five categories of urban green areas defined in Chapter 2, based on vegetation cover (grassland and forest), green space type, and levels of public use typically observed across the city. The five categories considered were grasslands in urban parks inside and outside the historic centre (UPC and UP, respectively), grasslands in small urban green islands (UGI), grasslands in peri-urban parks (PP), and forested areas within peri-urban parks (PPf). Three sites were chosen within each category, avoiding plots with extreme pH or SOC values and ensuring that the selected sites spanned the range of bulk density values observed for that category. Overall, this design aimed to capture the diversity of vegetation structure, management intensity, and land-use history across Milan's green spaces. For a detailed description of the study area, the categories of urban green areas, and other contextual information, see Chapter 2.



Figure 1. Location of the selected sampling sites ($n=15$). Sites are grouped by green area category, as indicated in the legend; numbers identify individual sampling sites within each category.

4.2.2 Data collection

4.2.2.1 Soil microarthropod sampling and QBS-ar index calculation

Sampling was conducted in October–November 2024; autumn represents a suitable period for soil microarthropod surveys, as soil moisture and temperature conditions are generally optimal for their activity (Menta et al., 2018a). Sampling order was arranged to minimize potential biases related to seasonal progression and weather variability: one site per green area category was sampled in rotation before proceeding to the next round. At each study site, samples were collected within the 4 × 4 m plot previously defined in Chapter 2. Within each plot, three soil cores (0–10 cm depth, approximately 1000 cm³ each) were collected, including the organic horizons and litter layer when present. The three samples were processed separately. In the laboratory, soil microarthropods were extracted using Berlese–Tullgren funnels equipped with a 3 mm mesh and a 60 W incandescent lamp for 15 days. Organisms were collected in a mixture of ethanol and glycerol (2:1) and subsequently examined under a stereomicroscope.

Microarthropods were assigned to a taxonomic group and to the corresponding biological form (BF) according to the QBS-ar protocol (Parisi et al., 2005). Each biological form received an Eco-Morphological Index (EMI), reflecting the degree of morphological specialization to edaphic life and ranging from 1 (low adaptation to soil life) to 20 (high adaptation). For each site, the fauna extracted from the three soil samples was analysed separately but considered jointly for the calculation of the index. The QBS-ar value was then obtained as the sum of the EMI scores assigned to all taxonomic groups recorded across the three samples. When multiple biological forms belonging to the same group were found, only the highest EMI value for that group was used in the calculation.

4.2.2.2 Characterization of soil chemical and physical properties

This study used the dataset on soil chemical and physical properties collected in Chapter 2. From the three soil layers sampled in Chapter 2, only data from the 0–10 cm layer were used, corresponding to the soil depth sampled for microarthropods. Composite soil samples (five subsamples per plot) were air-dried, sieved (< 2 mm), and analyzed for pH (1:2.5 soil/water), soil organic carbon (SOC, after carbonate removal), total nitrogen, available phosphorus (Olsen method), and particle-size distribution (sieving and sedimentation). Pseudo–total concentrations of heavy metals (Cr, Cu, Ni, Pb, Zn) were determined on the same soil samples as part of the earthworm study (Chapter 3), by acid digestion (HCl + HNO₃) followed by atomic absorption spectroscopy (AAS).

Bulk density (BD) was measured in the top 5 cm from three undisturbed soil cores (100 cm³ each), combined into a composite sample. Soil penetration resistance (PR) was measured in the field at 2.5 cm intervals using a penetrometer, with five repeated readings averaged per plot. To allow direct comparison

among measurements taken under different soil moisture conditions, PR data were corrected for water content, adjusting values to a common reference soil water content corresponding to field capacity. The volumetric content of rock fragments was also measured on the 0–10 cm soil cores collected for QBS-ar sampling.

4.2.2.3 Environmental parameters

At each site, soil temperature and soil water content (Time Domain Reflectometry, TDR) were measured concurrently with soil fauna sampling at two depths (0–5 and 10–15 cm). Measurements were taken in triplicate and averaged to obtain one value per depth and site.

In addition to these local soil variables, spatial variables were derived from GIS data layers using QGIS (v. 3.16.11). The distance of each plot from the city center (Piazza Duomo) was calculated as a proxy for the urbanization gradient.

4.2.3 Statistical analyses

Analyses were based on soil data from the 0–10 cm layer, except for bulk density, which was measured in the 0–5 cm layer only. Penetration resistance values were averaged over the 0–10 cm depth, to ensure consistency with the other soil properties. Soil temperature and moisture data, collected at two different depths, were also averaged to obtain a single representative value per site.

Basic descriptive statistics (mean, minimum, maximum, standard deviation) were computed for QBS-ar, as well as for the main physical and chemical soil properties, across the 15 sites.

Differences in QBS-ar and soil properties among the five green area categories (UPC, UP, UGI, PP, PPf) were tested with a one-way ANOVA. Residuals were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test). When significant differences were detected, post-hoc pairwise comparisons were performed using Tukey’s HSD test ($p < 0.05$).

Associations between QBS-ar and soil or environmental variables were quantified using Spearman’s rank correlation, as exploratory analysis indicated monotonic but non-linear relationships. Correlations were evaluated at $\alpha = 0.05$.

To explore multivariate patterns and visualize the joint structure of environmental gradients, a principal component analysis (PCA) was performed on standardized variables (centred and scaled), including QBS-ar among the active variables.

All statistical analyses were performed in R (version 4.5.0, R Core Team 2025) using the packages *stats*, *car*, *emmeans*, *Hmisc*, *FactoMineR*, and *factoextra*.

4.3 Results

Soil physical and chemical properties (Table 1) were consistent with the patterns previously observed in Chapter 2. Textures classes ranged from loam to sandy loam. SOC content was uniformly high across categories (mean \pm SD: 3.11 \pm 0.75 %), and C:N ratios were similarly homogeneous (range 10–12). Available phosphorus showed wide variability among plots (9–127 mg kg⁻¹; mean \pm SD: 26 \pm 15 mg kg⁻¹) but no significant differences among categories.

Table 1. Soil chemical and physical properties of the study sites. All soil properties refer to the 0–10 cm layer, except BD (0–5 cm layer). Mean and standard deviation are reported for each green area category.

| Plot | Text | BD g cm ⁻³ | PR MPa | pH | SOC % | N % | C:N | avP mg kg ⁻¹ | mg kg ⁻¹ | | | | |
|-------|-------------|--------------------------|-------------|------------|-------------|-------------|-----------|----------------------------|---------------------|------------|-----------|------------|------------|
| | | | | | | | | | Cr | Cu | Ni | Pb | Zn |
| PPf-1 | SL | 0.85 | 0.86 | 5.4 | 2.30 | 0.19 | 12 | 16 | 64 | 26 | 38 | 99 | 60 |
| PPf-2 | L | 0.85 | 0.66 | 4.9 | 2.69 | 0.23 | 12 | 18 | 75 | 36 | 37 | 140 | 62 |
| PPf-3 | L | 0.91 | 0.92 | 5.7 | 3.34 | 0.31 | 11 | 15 | 55 | 22 | 31 | 85 | 66 |
| | <i>mean</i> | 0.87 | 0.81 | 5.3 | 2.78 | 0.24 | 12 | 16 | 65 | 28 | 35 | 108 | 63 |
| | <i>SD</i> | 0.03 | 0.14 | 0.4 | 0.53 | 0.06 | 1 | 2 | 10 | 7 | 4 | 29 | 3 |
| PP-1 | L | 1.11 | 1.72 | 5.2 | 3.02 | 0.30 | 10 | 12 | 75 | 33 | 39 | 160 | 74 |
| PP-2 | SL | 1.17 | 1.13 | 5.8 | 2.09 | 0.21 | 10 | 9 | 56 | 15 | 27 | 56 | 41 |
| PP-3 | SL | 0.98 | 1.45 | 5.5 | 3.31 | 0.28 | 12 | 20 | 50 | 40 | 26 | 69 | 51 |
| | <i>mean</i> | 1.09 | 1.43 | 5.5 | 2.81 | 0.26 | 11 | 14 | 60 | 29 | 31 | 95 | 55 |
| | <i>SD</i> | 0.10 | 0.30 | 0.3 | 0.64 | 0.05 | 1 | 6 | 13 | 13 | 7 | 57 | 17 |
| UP-1 | SL | 1.02 | 1.88 | 5.7 | 2.99 | 0.28 | 11 | 9 | 65 | 24 | 48 | 65 | 60 |
| UP-2 | SL | 0.99 | 1.59 | 6.1 | 4.87 | 0.44 | 11 | 67 | 74 | 183 | 55 | 268 | 171 |
| UP-3 | SL | 1.03 | 2.07 | 6.6 | 4.09 | 0.41 | 10 | 22 | 91 | 133 | 76 | 182 | 125 |
| | <i>mean</i> | 1.01 | 1.85 | 6.1 | 3.99 | 0.37 | 11 | 33 | 77 | 113 | 60 | 172 | 119 |
| | <i>SD</i> | 0.02 | 0.24 | 0.5 | 0.94 | 0.09 | 1 | 31 | 13 | 81 | 15 | 102 | 55 |
| UGI-1 | L | 1.13 | 2.01 | 7.2 | 2.25 | 0.21 | 11 | 36 | 64 | 32 | 55 | 52 | 55 |
| UGI-2 | SL | 1.05 | 1.88 | 7.4 | 3.20 | 0.27 | 12 | 41 | 64 | 59 | 49 | 117 | 80 |
| UGI-3 | L | 1.17 | 1.99 | 7.3 | 2.82 | 0.24 | 12 | 26 | 61 | 46 | 49 | 85 | 79 |
| | <i>mean</i> | 1.12 | 1.96 | 7.3 | 2.76 | 0.24 | 11 | 34 | 63 | 45 | 51 | 85 | 72 |
| | <i>SD</i> | 0.06 | 0.07 | 0.1 | 0.48 | 0.03 | 1 | 8 | 2 | 14 | 3 | 32 | 14 |
| UPC-1 | SL | 1.24 | 1.79 | 6.8 | 3.12 | 0.29 | 11 | 39 | 90 | 97 | 49 | 226 | 131 |
| UPC-2 | SL | 1.27 | 2.06 | 7.3 | 2.59 | 0.24 | 11 | 26 | 77 | 59 | 45 | 123 | 73 |
| UPC-3 | SL | 1.16 | 2.27 | 6.9 | 3.92 | 0.36 | 11 | 28 | 78 | 151 | 54 | 361 | 183 |
| | <i>mean</i> | 1.22 | 2.04 | 7.0 | 3.21 | 0.29 | 11 | 31 | 82 | 102 | 49 | 237 | 129 |
| | <i>SD</i> | 0.06 | 0.24 | 0.3 | 0.67 | 0.06 | 0 | 7 | 7 | 46 | 5 | 119 | 55 |

Bulk density and penetration resistance showed considerable variation across sites (BD: 0.85–1.27 g cm⁻³; PR: 0.66–2.27 MPa), with significant differences among green area categories (ANOVA, $p < 0.001$; Fig. 2). PPf showed the lowest compaction levels (BD: mean = 0.87; 95 % CI [0.79–0.95] g cm⁻³; PR: mean = 0.81, 95% CI [0.54–1.09] MPa) whereas UPC exhibited the highest (BD: mean = 1.22; 95 % CI [1.15–1.30] g cm⁻³; PR: mean = 2.04, 95% CI [1.76–2.32] MPa per PR).

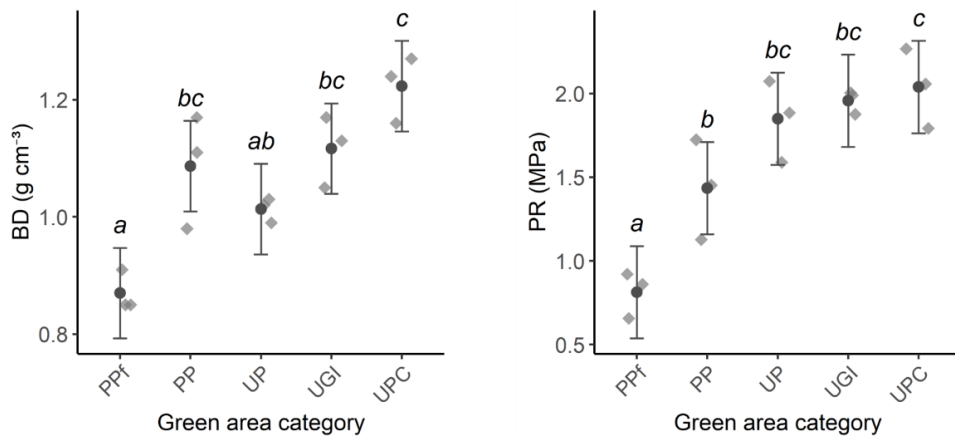


Figure 2. One-way ANOVA results for BD and PR across green area categories. Estimated marginal means ($\pm 95\%$ CI) are shown. Grey dots represent individual plot observations. Different letters indicate significant differences among categories (Tukey post hoc test).

pH ranged from 4.9 to 7.4, with significant differences among categories (ANOVA, $p < 0.001$; Fig. 3): PPf and PP showed the lowest values (PPf: mean = 5.3, 95 % CI [4.9–5.8]; PP: mean = 5.5, 95 % CI [5.1–5.9]), while UGI displayed the highest (mean = 7.0, 95 % CI [6.6–7.4]). Beyond statistical differences, this pattern reflects a broader opposition between peri-urban parks—characterised by more acidic conditions—and urban contexts (UGI, UP, UPC), where pH tended to be higher.

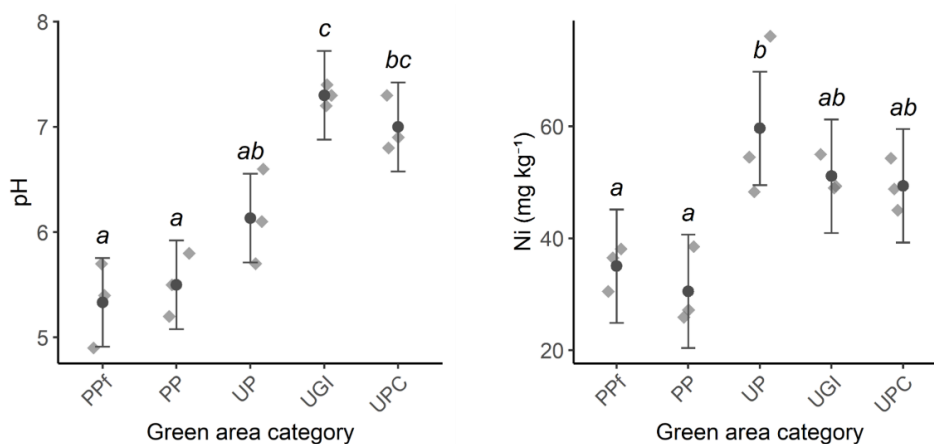


Figure 3. One-way ANOVA results for pH and Ni across green area categories. Estimated marginal means ($\pm 95\%$ CI) are shown. Grey dots represent individual plot observations. Different letters indicate significant differences among categories (Tukey post hoc test).

Heavy metal content varied substantially across sites. Only Ni differed significantly among categories (ANOVA, $p = 0.007$; Fig. 3), with maximum values in UP (mean = 60, 95 % CI [50–70] mg kg^{-1}) and minimum values in PPf and PP (e.g., PP: mean = 31, 95 % CI [20–41] mg kg^{-1}). Even in the absence of statistically significant differences, a clear gradient is evident, with urban parks (UP and UPC) at one end and peri-urban parks (PP and PPf) at the other, while UGI generally exhibited values comparable to those of peri-urban parks. This pattern is particularly evident for Pb, Cu, and Zn (Table 1). For instance, Pb averaged $95 \pm 57 \text{ mg kg}^{-1}$ in PP, with similar values in PPf and UGI (108 ± 29 and $85 \pm 32 \text{ mg kg}^{-1}$, respectively), but reached $237 \pm 119 \text{ mg kg}^{-1}$ in UPC and $172 \pm 102 \text{ mg kg}^{-1}$ in UP.

It is essential to note that the heavy metal concentrations reported here refer to the fine-earth fraction (<2 mm) of the surface layer (0–10 cm). They are not directly comparable with the threshold values established by the Italian Legislative Decree 152/2006, which require concentrations averaged over the entire soil profile (0–100 cm) and expressed on both fine-earth and gravel fractions. Therefore, any exceedances of regulatory limits in the fine fraction of the surface horizon do not constitute evidence of contamination under the law; rather, they may indicate the opportunity for further investigation in accordance with standardized protocols consistent with regulatory requirements.

Across the 15 sampled sites, QBS-ar values ranged from 61 to 136 (mean \pm SD: 88 ± 22) (Table 2). The lowest value was recorded in an urban park within the historical centre, whereas the highest was found in a peri-urban forest.

Table 2. QBS-ar values of the sampling sites, grouped by green area categories. Mean values and standard deviations for each category are reported.

| PPf | QBS-ar | PP | QBS-ar | UP | QBS-ar | UGI | QBS-ar | UPC | QBS-ar |
|-------------|--------|-------------|--------|-------------|--------|-------------|--------|-------------|--------|
| PPf-1 | 122 | PP-1 | 75 | UP-1 | 101 | UGI-1 | 93 | UPC-1 | 78 |
| PPf-2 | 136 | PP-2 | 71 | UP-2 | 82 | UGI-2 | 89 | UPC-2 | 61 |
| PPf-3 | 95 | PP-3 | 72 | UP-3 | 70 | UGI-3 | 111 | UPC-3 | 67 |
| <i>mean</i> | 118 | <i>mean</i> | 73 | <i>mean</i> | 84 | <i>mean</i> | 98 | <i>mean</i> | 69 |
| <i>SD</i> | 21 | <i>SD</i> | 2 | <i>SD</i> | 16 | <i>SD</i> | 12 | <i>SD</i> | 9 |

A total of 18 microarthropod groups were detected across the 15 sampled sites, with 5 to 10 taxa recorded per site (Table 3). Acari and Collembola (including the euedaphic form) were the only groups consistently found in all sites, followed by Formicidae, Diptera larvae, and other holometabolous insect (larvae or adults), which occurred in the majority of sites. In contrast, several taxa were recorded only occasionally, including Isopoda, Diplopoda, Pauropoda, Symphyla, Protura, Diplura, Orthoptera and Thysanoptera.

Nine euedaphic groups were identified in total (Table 3): Acari, Pauropoda, Symphyla, Protura and Diplura, and the euedaphic forms of Collembola (Onychiuridae), Chilopoda (Geophilomorpha), Orthoptera

(Grillidae) and Diplopoda (small size forms). Peri-urban forests (PPf) were the only category in which all euedaphic groups detected in this study (except Orthoptera) were represented. Three taxa—Diplopoda, Pauropoda and Protura—were found exclusively in these forest sites.

QBS-ar significantly differed among categories of urban green areas (ANOVA, $p = 0.007$) (Fig. 4). Peri-urban forests (PPf) exhibited the highest mean values (mean = 118; 95% CI [101–135]), significantly higher than UPC and PP (mean = 69, 95% CI [52–86] for UPC; mean = 73, 95% CI [56–90] for PP). UP and UGI exhibited intermediate values (mean = 84, 95% CI [67–102] for UP; mean = 98, 95% CI [81–115] for UGI).

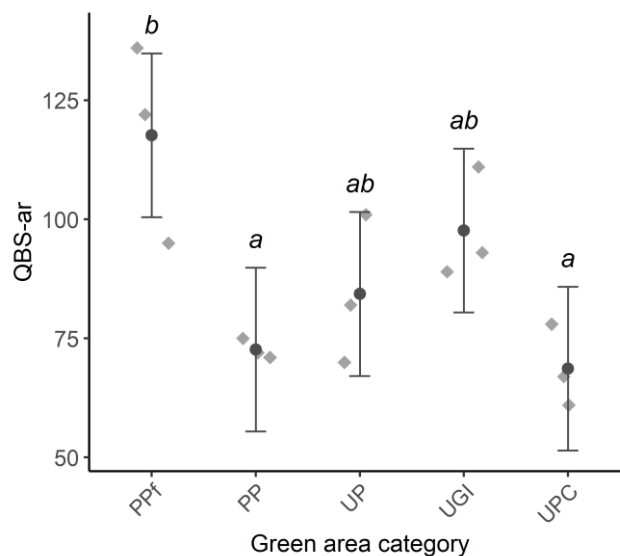


Figure 4. One-way ANOVA results for QBS-ar values across green area categories. Estimated marginal means ($\pm 95\%$ CI) are shown. Grey dots represent individual plot observations. Different letters indicate significant differences among categories (Tukey post hoc test).

Spearman's rank correlations indicated that QBS-ar was significantly related only to bulk density ($\rho = -0.56$, $p = 0.029$) and soil penetration resistance ($\rho = -0.53$, $p = 0.043$). A near-significant positive correlation was also observed with the C:N ratio ($\rho = 0.51$, $p = 0.054$).

The first two principal components of the PCA explained 37% and 15.3% of the total variance, respectively (Fig. 5). PC1 represented the main environmental gradient, broadly opposing peri-urban sites to urban areas. The axis was mainly driven by distance from the city centre (negative side) and, in the opposite direction, by higher pH, SOC, total nitrogen, available phosphorus, heavy metals, bulk density, and penetration resistance — distinguishing less-disturbed soils from more altered and compacted ones. PC2 represented a weaker secondary gradient, contrasting soils with higher SOC, N and metal content (positive loadings), with those characterised by higher pH and compaction (negative loadings).

The QBS-ar index was oriented toward the upper-left portion of the biplot (Fig. 5), being weakly to moderately negatively associated with soil compaction, higher pH, and the proportion of rock fragments

in the QBS-ar samples. It showed a positive association with the distance from the city centre and the C/N ratio. The short QBS-ar vector indicates a relatively weak relationship between QBS-ar and the measured environmental gradients. No spatial clustering of sites according to their QBS-ar values was observed, as high and low scores were evenly dispersed across the biplot space.

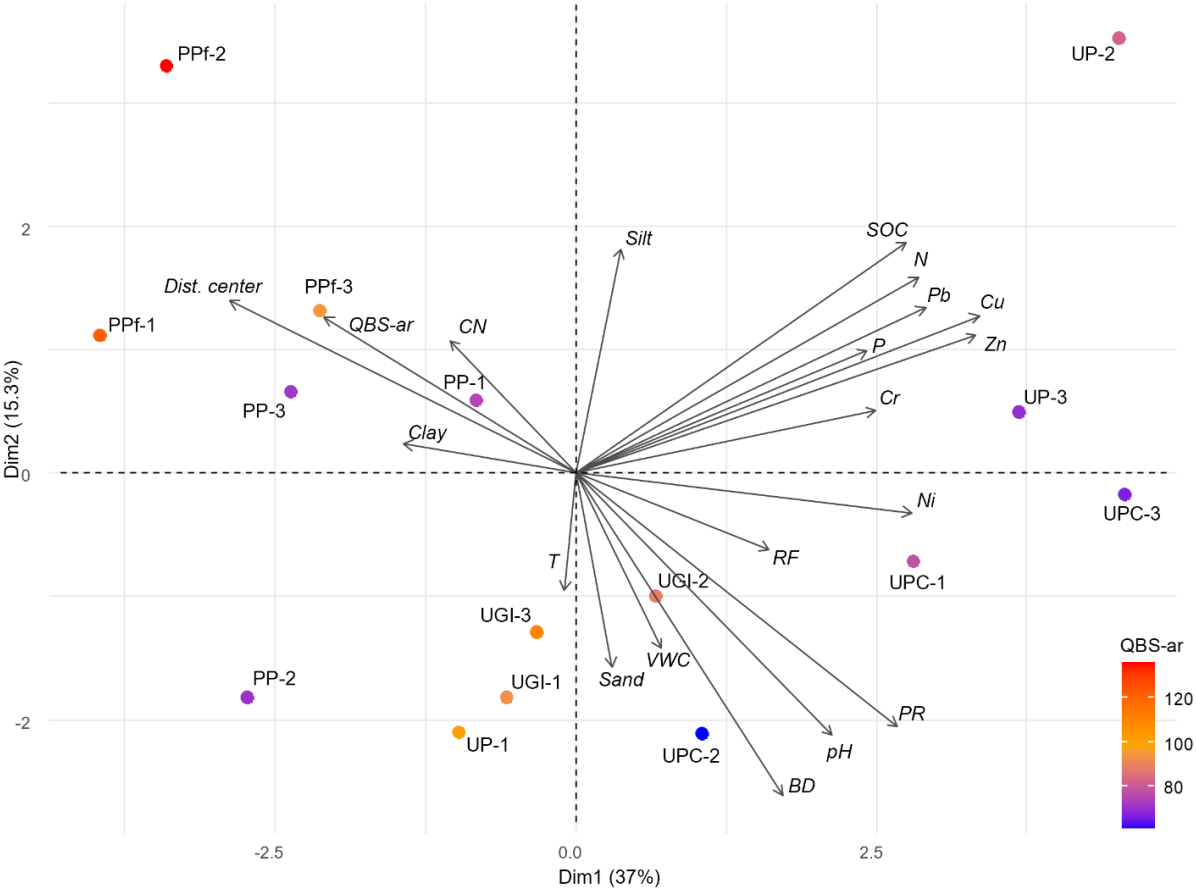


Figure 5. PCA biplot of soil chemical and physical properties, environmental variables and QBS-ar. Points represent individual plots, coloured by QBS-ar values; arrows indicate variable loadings. VWC: Volumetric Water Content. RF: rock fragments.

Table 3. Occurrence of QBS-ar taxonomic groups across individual plots, grouped by green area category. The EMI score range for each taxonomic group is reported. ✓ = presence of the taxonomic group; ★ = presence of euedaphic forms (EMI = 20). * only Formicidae were found.

| Taxonomic groups | EMI | PPf-1 | PPf-2 | PPf-3 | PP-1 | PP-2 | PP-3 | UP-1 | UP-2 | UP-3 | UGI-1 | UGI-2 | UGI-3 | UPC-1 | UPC-2 | UPC-3 |
|----------------------|-------|-------|-------|-------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|
| Pseudoscorpiones | 20 | | | | | | | | | | | | | | | |
| Scorpiones | 10 | | | | | | | | | | | | | | | |
| Palpigradi | 20 | | | | | | | | | | | | | | | |
| Opiliones | 10 | | | | | | | | | | | | | | | |
| Araneae | 1-5 | | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | | |
| Acari | 20 | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ |
| Isopoda | 10 | ✓ | | | | | | | | | | | | | | |
| Diplopoda | 10-20 | ✓ ★ | ✓ | ✓ | | | | | | | ✓ | | | | | |
| Paupoda | 20 | | ✓ ★ | | | | | | | | | | | | | |
| Symphyla | 20 | ✓ ★ | | | | | | | | | | | ✓ ★ | ✓ ★ | | |
| Chilopoda | 10-20 | | ✓ ★ | ✓ ★ | | ✓ | | | ✓ ★ | | ✓ | ✓ ★ | ✓ ★ | | | |
| Protura | 20 | ✓ ★ | | | | | | | | | | | | | | |
| Diplura | 20 | | ✓ ★ | | | | | ✓ ★ | | | | | | | | |
| Collembola | 1-20 | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ | ✓ ★ |
| Microcoryphia | 10 | | | | | | | | | | | | | | | |
| Zygentoma | 10 | | | | | | | | | | | | | | | |
| Dermaptera | 1 | | | | | | | | | | | | | | | |
| Orthoptera | 1-20 | | | | | | | ✓ ★ | | | | | | | | |
| Embioptera | 10 | | | | | | | | | | | | | | | |
| Isoptera | 10 | | | | | | | | | | | | | | | |
| Blattaria | 5 | | | | | | | | | | | | | | | |
| Psocoptera | 1 | ✓ | | | | | | ✓ | ✓ | | ✓ | ✓ | | ✓ | ✓ | ✓ |
| Hemiptera | 1-5 | | | | | | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | | ✓ |
| Thysanoptera | 1 | ✓ | ✓ | | | | | | | | | | | | | |
| Hymenoptera* | 1-5 | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ |
| Diptera (larvae) | 10 | | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Holometabola (other) | 1-10 | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ |
| Coleoptera | 1-20 | | | | ✓ | ✓ | ✓ | | | | ✓ | ✓ | ✓ | | | |
| N. groups | | 9 | 10 | 5 | 6 | 7 | 8 | 8 | 9 | 6 | 10 | 10 | 9 | 8 | 5 | 7 |
| QBS-ar | | 122 | 136 | 95 | 75 | 71 | 72 | 101 | 82 | 70 | 93 | 89 | 111 | 78 | 61 | 67 |

4.4 Discussion

A systematic evaluation of soil microarthropods and soil biological quality had so far been lacking for Milan's urban green spaces. Here, we addressed this gap by applying the QBS-ar index across a wide range of green-area categories and by examining its relationships with soil properties, land use, management intensity and site-specific histories. Building on our previous assessment of the physical and chemical characteristics of Milan's soils, this study adds a complementary biological dimension, contributing to a more integrated view of soil conditions in the city. Approaches that combine multiple dimensions of the soil ecosystem within the same urban context remain relatively uncommon (e.g., Joimel et al., 2016, 2017; Horváth et al., 2021).

Our previous assessment of chemical and physical properties (Chapter 2) showed that soils in Milan's green spaces were generally in good ecological condition: compaction levels were low, SOC content was consistently high and pH remained within a favourable ecological range. Urban pressure, however, left a recognisable imprint on several parameters, with bulk density, penetration resistance and pH following a clear urban–peri-urban gradient. Peri-urban areas retained more semi-natural features, whereas urban areas—particularly UPC and UGI—showed stronger signs of alteration, reflecting differences in management intensity, recreational pressure and land-use history. These contrasts were especially evident in the topsoil.

In the subset of sites analysed for soil fauna, soil properties broadly reflected these patterns, although the gradient were less continuous. Bulk density and penetration resistance still showed clear extremes—lowest in PPf and highest in UPC—yet values remained well below critical thresholds, as already discussed in Chapter 2. These contrasts likely reflect differences in trampling and maintenance intensity, and, in forests, the buffering effect of the litter layer. pH followed a similar pattern: lower values characterised peri-urban parks, both grasslands and forests, whereas higher values occurred in UGI, UP and UPC, probably due to calcareous fill materials or fertilisation in more intensively managed areas. Despite this variability, the overall pH range (from acidic to slightly alkaline) remained within levels generally tolerated by soil microarthropods (Van Straalen and Verhoef, 1997). As in the broader survey, SOC was uniformly high across all categories, a pattern consistent with land-use legacies: the forest sites are relatively recent plantings on former agricultural soils, which aligns with the only slightly higher C:N ratios observed in PPf. Heavy metal concentrations were generally higher in UP and UPC than in PP and PPf, whereas UGI showed a distinct profile linked to their specific management histories.

QBS-ar showed significant negative associations with bulk density and penetration resistance, and a weak positive relationship with the C:N ratio. Similar patterns have been widely reported in urban environments, where compaction and the amount and quality of litter inputs are key drivers shaping edaphic microarthropod communities (Ungaro et al., 2022; Gardini et al., 2025).

In contrast to findings from agricultural systems (Menta et al., 2018b) and some urban studies (Magro et al., 2013), no association emerged with SOC, likely because organic matter content in our topsoils was relatively high and showed little variation. Likewise, although heavy metals are often cited as factors associated with reduced microarthropod diversity in urban soils (Santorufó et al., 2012; Horváth et al., 2021; Tóth et al., 2023), no effects were detected in our sites.

Overall, QBS-ar values in Milan (61–136, mean 88) indicate a generally moderate soil biological quality. Only one third of the sampled sites exceeded the threshold of 94 proposed by Menta et al. (2018a) to classify soils as biologically “good”. However, these values are comparable to those typically reported for Italian cities, although they tend to be slightly lower on average. For example, Ungaro et al. (2022) reported QBS-ar values ranging from 56 to 182 (mean 101) in Carpi, while Santorufó et al. (2012) found values between 78 and 145 (mean 106) in Naples. By contrast, exceptionally high scores have been documented in Rome (107–219, mean 168; Gardini et al., 2025). As expected, QBS-ar values in Milan were higher than those reported for arable land (mean 76 ± 24 ; Aspetti et al., 2010) but remained markedly lower than those observed in natural grasslands and woodlands in the Po Plain (140 ± 12 and 172 ± 17 , respectively; Menta et al., 2011).

Differences in QBS-ar emerged across green areas categories. Peri-urban forests showed the highest values, whereas the lowest scores occurred in UPC and, unexpectedly, in PP. UP and UGI displayed intermediate conditions, with considerable site-to-site variability. Notably, PPf were the only category exceeding the threshold of 94 on average.

Forests are consistently reported as the habitats with the highest QBS-ar values within urban environments (Tóth et al., 2023; Gardini et al., 2025). PPf sites were characterised by low bulk density and penetration resistance, minimal trampling, and the presence of a well-developed, undisturbed litter layer, which provides food resources and creates favourable microhabitats by buffering soil temperature and moisture (Maisto et al., 2016). Such conditions are known to support specialised and structurally complex microarthropod communities (Huang et al., 2020), and indeed PPf hosted several euedaphic and disturbance-sensitive taxa (e.g. Pauropoda, Protura, Diplura), in line with findings from other urban forests (Rodríguez -Pajares et al., 2025). The weak positive correlation observed with C:N likely reflected the broad ecological role of litter for microarthropods rather than substantive differences in organic-matter quality, given the limited variation in C:N across categories.

In contrast, the lowest QBS-ar values were recorded in grasslands within central urban parks. These sites have been subjected to long-standing and intense urban pressure, which is reflected—among other aspects—in their relatively higher compaction levels, consistent with the negative correlations observed between QBS-ar and both BD and PR. In addition, the combined effects of trampling and the routine removal of fallen leaves and mowing residues likely limit the development of surface organic horizons, resulting in less favourable

topsoil conditions for microarthropods. This pattern is consistent with broader evidence that higher compaction, disturbance and management intensity levels negatively affect soil microarthropod communities in urban green spaces (Proske et al., 2022; Ungaro et al., 2022). Higher heavy metal concentrations in UPC did not appear to contribute to their low QBS-ar values, as the index showed no association with metal content in our dataset.

More unexpected were the low QBS-ar scores observed in peri-urban grasslands, despite minimal trampling, reduced management and physical and chemical soil properties resembling those of semi-natural grasslands. This mismatch indicates that favourable physical and chemical conditions do not necessarily translate into high biological soil quality in urban contexts (Joimel et al., 2017), and suggests that unmeasured, site-specific factors may have influenced microarthropod communities.

Intermediate QBS-ar values were recorded in UP, consistent with the wide range of land-use histories, management regimes and levels of recreational pressure characterising this category.

UGI showed surprisingly high QBS-ar values, ranking immediately after forest sites. These small, isolated patches embedded in the urban matrix and subjected to intensive management, are characterised by soil properties that clearly reflect disturbance, as also observed in similar contexts (Huang et al., 2020; Ungaro et al., 2022). A low biological quality—comparable to that of UPC—would therefore have been expected.

The high QBS-ar values observed in UGI are likely related to the origin of their topsoil, as suggested by heavy metal concentrations. Compared to UP and UPC, in fact, these sites showed markedly lower concentrations of Pb—a long-term marker of past traffic emissions (Manta et al., 2002; Calace et al., 2012)—with values even comparable to those of peri-urban areas; Zn and Cu, markers of current traffic, followed a similar though less pronounced trend. Given the proximity of UGI to high traffic roads, such metal patterns suggest that these soils do not have a long urban history but likely received external inputs from less disturbed contexts. The microarthropod community introduced with this material was probably already rich and structured and could likely persist even under moderate disturbance (Huang et al., 2020). Site histories support this interpretation, as available information indicates recent management interventions involving soil replacement or superficial restyling in two of these areas. Such interventions are relatively common in small urban islands, which—because of their size and ornamental role—are more likely to undergo substantial restyling and topsoil additions, practices far less feasible in larger parks. These findings highlight the intrinsic unpredictability of these small patches and shows that management histories and soil input events can override typical urban pressure gradients, underscoring the need to account for site-specific histories when interpreting soil fauna patterns in urban environments (Guilland et al., 2018).

While the differences in QBS-ar values observed in forests (PPf) and central urban parks (UPC) were coherent with their different site characteristics in terms of vegetation structure, land use and disturbance intensity

(Gardini et al., 2025), other patterns were less straightforward. In particular, peri-urban grasslands (PP) and lawns in UPC displayed similar QBS-ar values despite evident differences in disturbance levels and soil conditions. This suggests that the index— although effective in capturing pronounced ecological contrasts among broad habitat types (Maisto et al., 2017)—may be less sensitive to finer-scale variations in management, disturbance or soil properties. Similar concerns have been raised in agricultural settings, where QBS-ar sometimes failed to detect subtle differences in management intensity (Naglič et al., 2025).

The multivariate analysis provides useful context for this interpretation. Although it revealed a clear urban–peri-urban gradient in soil conditions—driven primarily by compaction, pH, SOC and metal content—QBS-ar responded only weakly to this dominant environmental structure. The biplot showed no clustering of sampling sites according to their QBS-ar values, indicating that the index captures only a limited portion of the overall variability.

An element likely contributing to this weak alignment is the intrinsic heterogeneity and unpredictability of urban soils. In cities, microarthropod communities often do not follow soil chemical and physical gradients in a consistent way (Joimel et al., 2017). Site-specific factors, such as legacy effects or recent site interventions, may lead to biological responses that diverge from general soil trends. Such processes, typical of urban contexts (Hazelton and Murphy, 2021), can therefore reduce the degree to which QBS-ar reflects environmental gradients, independently of any intrinsic limitation of the index.

It is also important to note that soils in our study generally fell within favourable conditions for edaphic fauna. In the absence of strong environmental constraints, QBS-ar is likely to respond primarily to broad habitat features—such as the presence or absence of a stable litter layer—rather than to finer-scale variation in soil conditions or management.

Overall, based on our results, QBS-ar does not seem to provide an integrated picture of the environmental drivers and urban pressures shaping soils in the city, and its interpretation as a general indicator of green space soil status remains uncertain. Its usefulness as a proxy for other soil attributes also appears limited, as these can be more directly and reliably assessed through standard measurements. Nonetheless, the relatively small number of sites investigated may have influenced these outcomes, and our conclusions should be viewed with appropriate caution. By contrast, QBS-ar remains particularly useful for capturing, in a concise and accessible way, the condition of soil microarthropod communities. In this sense, it offers a rapid indication of biological soil quality and of how diversified—or simplified—microarthropod assemblages are within the urban environment.

Peri-urban forests clearly emerged as the most favourable habitats for soil microarthropods, hosting several disturbance-sensitive and euedaphic taxa. This underlines the crucial role of forests in sustaining soil

biodiversity within the city (Gardini et al., 2025). These areas should therefore be regarded as priority sites in urban planning and management.

Our findings also highlighted how local histories and discontinuous urban processes can shape biological outcomes in unexpected ways. Past interventions, such as soil replacement, may leave persistent biological legacies, underscoring the importance of accounting for site-specific trajectories in urban ecology (Pavao-Zuckerman, 2012). The role of soil replacement in shaping edaphic fauna—also relevant to restoration ecology—still requires specific investigation. These small patches would particularly benefit from temporal monitoring to assess whether the observed communities remain stable over time or decline as urban disturbance continues.

This study has limitations that should be acknowledged. The number of sites was relatively small, which restricts broad generalisations, and sampling was conducted in a single season, which is especially important considering the documented seasonal variability of QBS-ar (Santorufo et al., 2014). Nevertheless, values tend to remain comparable across years when sampling is performed during the same period (Aspetti et al., 2010), suggesting that our dataset could provide a reliable a reliable representation of local conditions.

4.5 Conclusions

Taken together, our results indicate that the biological quality of Milan's urban soils is generally moderate and broadly comparable to that reported for other Italian cities. QBS-ar proved valuable for describing the condition and diversity of microarthropod communities, whereas its usefulness as a proxy for other environmental factors appears limited. Peri-urban forests emerged as key habitats for disturbance-sensitive taxa, confirming their central role in maintaining soil biodiversity, while the contrasting behaviour of UGI emphasised the influence of local site-specific histories on edaphic fauna.

From a management perspective, the results highlight the importance of practices that preserve surface organic layers and limit excessive compaction, contributing to preserving microhabitat quality for edaphic fauna (Blumlein et al., 2012). In forested areas, retaining an undisturbed litter layer is particularly beneficial, while in grasslands reducing the systematic removal of fallen leaves or mowing residues—where compatible with other uses—can help maintain suitable topsoil conditions.

QBS-ar may also serve as a simple and repeatable tool for monitoring biological soil quality over time, especially in sites where the persistence of microarthropod communities is uncertain.

Overall, this study provides a first baseline for microarthropod-based assessments in Milan's urban soils and underscores the value of integrating biological indicators and physical and chemical properties to capture the complexity of urban soil systems.

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Supplementary Materials

ENVIRONMENTAL AND SPATIAL VARIABLES ACROSS THE STUDY SITES

Notes: Dist. Center: distance from the city center; T: soil temperature; VWC: soil volumetric water content.

| Plot | Dist. Center | T | VWC |
|-------------|---------------------|----------|------------|
| | km | °C | % |
| PPf-1 | 9.13 | 9.2 | 20 |
| PPf-2 | 8.78 | 9.4 | 18 |
| PPf-3 | 7.84 | 17.6 | 29 |
| PP-1 | 8.50 | 10.1 | 27 |
| PP-2 | 7.07 | 10.5 | 30 |
| PP-3 | 8.47 | 17.9 | 24 |
| UP-1 | 5.43 | 18.6 | 32 |
| UP-2 | 1.86 | 8.9 | 30 |
| UP-3 | 5.38 | 13.8 | 19 |
| UGI-1 | 2.42 | 9.4 | 28 |
| UGI-2 | 3.25 | 18.6 | 20 |
| UGI-3 | 2.46 | 17.9 | 28 |
| UPC-1 | 1.30 | 16.9 | 31 |
| UPC-2 | 1.59 | 8.2 | 25 |
| UPC-3 | 1.11 | 11.2 | 27 |

ANOVA RESULTS FOR QBS-ar ACROSS GREEN AREA CATEGORIES

Notes: PPf: peri-urban forest, PP: peri-urban park, UP: urban park, UGI: urban green island, UPC: urban park centre.

Model: QBSar ~ green_area

Residuals:

| Min | 1Q | Median | 3Q | Max |
|---------|--------|--------|-------|--------|
| -22.667 | -6.167 | -1.667 | 6.833 | 18.333 |

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) | |
|---------------|----------|------------|---------|----------|-----|
| (Intercept) | 72.667 | 7.723 | 9.409 | 2.77e-06 | *** |
| green_areaPPf | 45.000 | 10.922 | 4.120 | 0.00208 | ** |
| green_areaUGI | 25.000 | 10.922 | 2.289 | 0.04509 | * |
| green_areaUP | 11.667 | 10.922 | 1.068 | 0.31054 | |
| green_areaUPC | -4.000 | 10.922 | -0.366 | 0.72182 | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 13.38 on 10 degrees of freedom

Multiple R-squared: 0.7279, Adjusted R-squared: 0.6191

F-statistic: 6.688 on 4 and 10 DF, p-value: 0.006918

CHAPTER 5

SOIL WATER INFILTRATION

Abstract

In urban environments, soils in green spaces may contribute to the overall permeability of cities and have the potential to regulate surface runoff and flood risk at the city scale, yet urban soil infiltration remains poorly understood.

This chapter examined water infiltration as a key soil process in public green spaces in Milan. Water infiltration rates were quantified and their variability among different urban and peri-urban green area categories was evaluated. In addition, the role of soil properties, particularly soil compaction, and earthworm communities in controlling water infiltration was explored. The study was conducted at a subset of 15 sites selected from those investigated in Chapter 2.

Infiltration rates varied widely across sites, ranging from 15 to 288 mm h⁻¹ (mean ± SD: 134 ± 94 mm h⁻¹), and overall fell within a moderate to high range. The absence of significant differences among green area categories indicated that infiltration rates in Milan's urban soils were primarily controlled by site-specific soil conditions rather than by land use or vegetation type per se. When interpreted in relation to local precipitation regimes, observed infiltration rates suggest that soils in Milan's green spaces are generally able to infiltrate typical rainfall commonly experienced in the city, whereas the highest intensities recorded over time may exceed infiltration capacity. Urban parks in the city center, characterised by lower infiltration rates (mean ± SD: 40 ± 19 mm h⁻¹) and limited vegetation buffering, emerged as the most vulnerable green spaces with respect to high-intensity rainfall events.

Soil compaction (expressed by bulk density), sand content and earthworm biomass were the main drivers of water infiltration. Overall, the results emphasized that infiltration depended on the combined contribution of relatively stable soil attributes (such as texture), dynamic physical conditions (such as compaction) and soil biota. In particular, earthworms emerged as active contributors to infiltration in urban soils, a context where empirical evidence is still scarce.

5.1 Introduction

In urban areas, where sealed surfaces predominate, the capacity of the remaining unsealed soils to infiltrate rainwater is essential for reducing surface runoff and flood risk (Burghardt et al., 2015; Ren et al., 2020). This function is becoming increasingly important under climate change, which is expected to intensify the frequency and magnitude of extreme rainfall events (Müller et al., 2024). Despite its importance, water infiltration in urban soils remains comparatively underexplored (Yang and Zhang, 2011; Wang et al., 2018). The high heterogeneity and complexity of urban soils, resulting from diverse land use histories, disturbance regimes and management practices (Pouyat et al., 2020), make their hydrological behaviour difficult to predict (Whitehead et al., 2021), underscoring the need for further investigations aimed at characterizing infiltration under real urban conditions (Galli et al., 2021).

Available evidence nonetheless indicates that soils in urban green areas can play a key role in promoting infiltration, often being capable of infiltrating most rainfall events (Díaz-Sanz et al., 2020; Ungaro et al., 2022), although extreme rainfall may exceed their infiltration capacity (Phillips et al., 2019). This function is shared across a wide range of green spaces, from small and marginal elements such as green sidewalks and roundabouts (Galli et al., 2021), to lawns in urban parks (Whitehead et al., 2021) and to urban forests (Wang et al., 2018; Phillips et al., 2019). Together, these spaces form what is commonly referred to as urban green infrastructure (Mander et al., 2018). Accordingly, stormwater management strategies increasingly aim to enhance diffuse infiltration across urban areas by preserving the infiltration capacity of existing green spaces and by expanding vegetated cover in the city (Gill et al., 2007; Elliott et al., 2018). This approach moves beyond strategies relying exclusively on large, engineered infrastructures such as detention basins.

However, not all urban soils are able to sustain this function: in highly compacted soils, very low infiltration rates have been reported (Gregory et al., 2006; Yang and Zhang, 2011). Compaction is widely recognized as the main factor controlling infiltration in urban soils (Wang et al., 2018; Galli et al., 2021; Paradelo et al., 2025), together with soil texture and the presence of gravel or coarser fragments (Phillips et al., 2019). This is particularly relevant because, although compaction is not a universal feature of urban soils, it is nonetheless a relatively common condition and, more importantly, a dynamic process to which virtually all urban soils may be exposed over time (Burghardt et al., 2015; Pouyat et al., 2020).

Soil biota—and particularly earthworms—can play an active role in improving soil structure and porosity, thereby counteracting some of the negative effects of compaction (Edwards and Arancon, 2022). Through their burrowing activity, earthworms create networks of interconnected macropores that effectively enhance water movement through the soil profile (Capowiez et al., 2009; Fischer et al., 2014). The contribution of earthworms to infiltration varies across their ecological categories, reflecting differences in burrowing behaviour and gallery morphology (Shipitalo and Le Bayon, 2004; Hoeffner et al., 2022). Anecic earthworms,

which construct deep and permanent vertical burrows open to the soil surface, are generally considered the most effective in promoting preferential flow (Fischer et al., 2014; Andriuzzi et al., 2015). Importantly, earthworm burrows have been shown to be more resistant to mechanical compaction than other types of macropores (Shipitalo and Le Bayon, 2004). At the same time, however, earthworms are themselves negatively affected by soil compaction (Beylich et al., 2010), with high bulk density often associated with reduced abundance and biomass in urban soils (Smetak et al., 2007). Most of the current understanding of earthworm-mediated infiltration derives from agricultural systems (Capowiez et al., 2009; Wöhl et al., 2024). In contrast, much less is known about the contribution of earthworms to water infiltration in urban soils.

Building on the integrated assessment of soil physical and chemical properties and soil fauna presented in the previous chapters, this study examines water infiltration as a key soil process in urban environments. The study was conducted in public green spaces in Milan, including sites differing in soil properties, management intensity, disturbance levels and vegetation type (grassland and forest). To our knowledge, a comparative assessment of surface water infiltration across multiple different types of urban green spaces in Milan is still lacking. The chapter aims to quantify water infiltration rates and explore how infiltration patterns vary across the urban contexts, in order to evaluate the potential of soils in urban green spaces to infiltrate rainfall. In addition, it investigates the physical and biological factors controlling water infiltration in urban soils, with particular attention to the role of soil compaction and earthworms.

5.2 Materials and methods

5.2.1 Study area and sampling design

The study was conducted in public green spaces across the city of Milan. Infiltration measurements were carried out during the same field campaign as the earthworm and microarthropod surveys, at the same 15 sites (Fig. 1). These sites constitute a subset of those analysed in Chapter 2, in which the physical and chemical properties of Milan's soils were characterized. Sites were selected to represent the five categories of urban green areas defined in Chapter 2, which were based on vegetation cover (grassland and forest), green space type, and levels of public use typically observed across the city. The categories included: grasslands in urban parks inside and outside the historic centre (UPC and UP, respectively), grasslands in small urban green islands (UGI), grasslands in peri-urban parks (PP), and forested areas within peri-urban parks (PPf). Within each category, three sites were selected, representative of the most widespread textural classes in the study area (loam to sandy loam) and spanning the range of bulk density values observed within each category; plots with extreme SOC values were avoided. Overall, this design aimed to capture the diversity of vegetation structure,

management intensity, and land-use history that characterizes Milan's green spaces. A detailed description of the study area and site categories is provided in Chapters 2.



Figure 1. Location of the selected sampling sites ($n=15$). Sites are grouped by green area category, as indicated in the legend; numbers identify individual sampling sites within each category.

5.2.2 Data collection

5.2.2.1 Water infiltration measurements

Water infiltration was measured within the 4×4 m experimental plots previously defined. Measurements were performed using a single-ring infiltrometer (inner diameter = 18.7 cm; one repetition per plot). The ring was inserted a few centimetres into the soil to minimise lateral flow. Known water volumes (233 mL), corresponding to an initial water depth of 8.5 mm, were sequentially added into the ring, and the time required for each water increment to infiltrate was recorded. Field infiltration data were fitted using both exponential and hyperbolic functions, and the function providing the best fit to the observed data was used to derive the steady-state infiltration rate for each plot.

5.2.2.2 Characterization of soil chemical and physical properties

This study used the dataset on soil chemical and physical properties collected in Chapter 2. From the three soil layers sampled in Chapter 2, only data from the 0–10 and 10–20 cm layers were used. This choice ensures consistency with earthworm sampling depth and reflects the fact that surface infiltration is mainly influenced by topsoil characteristics. Composite soil samples (five subsamples per plot) were air-dried, sieved (< 2 mm),

and analyzed for pH (1:2.5 soil/water), soil organic carbon (SOC, after carbonate removal), total nitrogen, available phosphorus (Olsen method), and particle-size distribution (sieving and sedimentation).

Bulk density (BD) was measured in the top 5 cm from three undisturbed soil cores (100 cm³ each), combined into a composite sample. Soil penetration resistance (PR) was measured in the field at 2.5 cm intervals using a penetrometer, with five repeated readings averaged per plot. To allow direct comparison among measurements taken under different soil moisture conditions, PR data were corrected for water content, adjusting values to a common reference soil water content corresponding to field capacity (see Chapter 2 for methodological details).

5.2.2.3 Earthworm sampling, identification and community metrics

Earthworm sampling was carried out at the same time with infiltration measurements. The survey took place in October–November 2024, a period of peak activity for temperate earthworm communities (ISO 23611-6). At each study site, sampling took place within the 4 × 4 m plot. Four soil blocks (25 × 25 cm, 20 cm depth) were extracted per plot and hand-sorted to collect earthworms, which were then preserved in 70% ethanol. In the laboratory, earthworms were counted, individually weighed (fresh weight with gut content), assigned to a developmental stage (juvenile, sub-adult and adult), identified to species or subspecies level when possible, and classified into ecological categories (epigeic, endogeic and anecic; Bouché, 1972). Abundance (individuals m⁻²) and biomass (g m⁻²) were estimated for each plot by multiplying field data by 16, to obtain an estimation per square meter. Additional methodological details are provided in Chapter 3.

5.2.2.4 Environmental parameters

At each site, soil temperature and soil water content (Time Domain Reflectometry, TDR) were measured concurrently with soil fauna sampling at two depths (0–5 and 10–15 cm). Measurements were taken in triplicate and averaged to obtain one value per depth and site.

In addition to these local soil variables, spatial variables were derived from GIS data layers using QGIS (v. 3.16.11). The distance of each plot from the city center (Piazza Duomo) was calculated as a proxy for the urbanization gradient.

5.2.3 Statistical analyses

Statistical analyses were based on soil data from the 0–20 cm layer, except for bulk density, which was measured in the 0–5 cm layer only. Soil properties measured separately in the 0–10 and 10–20 cm layers were averaged to obtain a single value per site, while penetration resistance values, recorded at 2.5 cm intervals, were averaged over the same depth interval. Soil temperature and volumetric water content (0–5 and 10–15 cm) were averaged to obtain one site-level value.

Basic descriptive statistics (mean, minimum, maximum, SD) were computed across the 15 sites for infiltration rates, earthworm biomass and abundance, and the main physical and chemical soil properties.

Differences in infiltration rates among the five green area categories (UPC, UP, UGI, PP, PPf) were tested using a one-way ANOVA. Residuals were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test).

To investigate how infiltration rate relates to soil properties, earthworm metrics, and environmental variables, an initial screening was carried out by retaining as candidate predictors those variables showing at least a moderate association with infiltration (pairwise Pearson correlations, $|r| > 0.30$). Candidate predictors were then checked for collinearity and for each pair with $|r| > 0.7$, one variable was excluded (Dormann et al., 2013). Variable selection combined statistical screening with ecological relevance, measurement robustness, and the ability of predictors to meet linear-model assumptions. A linear model (LM) was thus fitted to assess the influence of the selected predictors on infiltration rate. All continuous variables were standardized prior to analysis. Model assumptions were evaluated through visual inspection of residual plots and using the Shapiro–Wilk test for normality and the Breusch–Pagan test for homoscedasticity. Residual spatial autocorrelation was assessed using Moran’s I.

All statistical analyses were performed in R (version 4.5.0; R Core Team 2025) using the packages *stats*, *car*, *lmtest*, *spdep*.

5.3 Results

Soils in the 0-20 cm layer (Table 1) had loam or sandy loam texture, and organic carbon content ranged from 1.51 to 4.04% (mean \pm SD: $2.37 \pm 0.68\%$). Bulk density in the upper 5 cm ranged from 0.85 to 1.27 g cm⁻³ (mean \pm SD: 1.06 ± 0.13 g cm⁻³), while penetration resistance (0–20 cm) varied between 0.85 and 2.92 MPa (mean \pm SD: 2.00 ± 0.62 MPa). Earthworm biomass ranged from 26.1 to 332.8 g m⁻² across sites (mean \pm SD: 128.4 ± 83.0 g m⁻²); anecic earthworms represented 71% of total biomass, followed by endogeic (28%) and epigeic (1%) (see also Chapter 3).

Infiltration rate varied widely across sites, ranging from 15 to 288 mm h⁻¹ (mean \pm SD: 134 ± 94 mm h⁻¹) (Table 1). It did not differ significantly among green area categories (ANOVA, $p > 0.05$; see Supplementary Materials). Nevertheless, UPC sites tended to show lower infiltration rates and limited variability (22–59 mm h⁻¹), whereas the other categories generally exhibited higher and markedly more variable infiltration rates. One PP site, however, recorded an exceptionally low value (15 mm h⁻¹), comparable to those observed in UPC. Overall, the broad within-category variability resulted in substantial overlap among groups.

Table 1. Infiltration rates, soil properties, earthworm metrics and environmental variables across the 15 sampling sites. Soil properties: 0–20 cm layer, except for BD (0–5 cm). VWC: Volumetric Water Content; EW ab: earthworm abundance EW bio: earthworm biomass.

| Plot | Inf. rate mm h ⁻¹ | VWC % | BD g cm ⁻³ | PR MPa | Sand g kg ⁻¹ | Silt g kg ⁻¹ | Clay g kg ⁻¹ | SOC % | EW ab. ind m ⁻² | EW bio. g m ⁻² | Dist. center km |
|-------|---------------------------------|----------|--------------------------|-----------|----------------------------|----------------------------|----------------------------|----------|-------------------------------|------------------------------|--------------------|
| PPf-1 | 247 | 20 | 0.85 | 1.10 | 600 | 287 | 114 | 1.57 | 192 | 56 | 9.1 |
| PPf-2 | 172 | 18 | 0.85 | 0.85 | 470 | 421 | 110 | 1.90 | 192 | 74 | 8.8 |
| PPf-3 | 90 | 29 | 0.91 | 0.99 | 441 | 453 | 107 | 2.79 | 508 | 231 | 7.8 |
| PP-1 | 15 | 27 | 1.11 | 2.06 | 445 | 436 | 120 | 2.03 | 68 | 26 | 8.5 |
| PP-2 | 101 | 30 | 1.17 | 1.61 | 558 | 360 | 83 | 1.51 | 396 | 159 | 7.1 |
| PP-3 | 285 | 24 | 0.98 | 1.93 | 528 | 405 | 68 | 2.56 | 840 | 333 | 8.5 |
| UP-1 | 245 | 32 | 1.02 | 2.37 | 592 | 327 | 82 | 2.03 | 352 | 108 | 5.4 |
| UP-2 | 93 | 30 | 0.99 | 2.01 | 439 | 481 | 81 | 4.04 | 396 | 119 | 1.9 |
| UP-3 | 288 | 19 | 1.03 | 2.47 | 554 | 346 | 101 | 2.75 | 316 | 121 | 5.4 |
| UGI-1 | 171 | 28 | 1.13 | 2.45 | 484 | 365 | 152 | 1.67 | 1104 | 253 | 2.4 |
| UGI-2 | 121 | 20 | 1.05 | 2.07 | 557 | 352 | 92 | 2.82 | 320 | 79 | 3.3 |
| UGI-3 | 68 | 28 | 1.17 | 2.92 | 483 | 430 | 87 | 2.01 | 448 | 104 | 2.5 |
| UPC-1 | 40 | 31 | 1.24 | 2.17 | 511 | 430 | 60 | 2.64 | 380 | 80 | 1.3 |
| UPC-2 | 22 | 25 | 1.27 | 2.45 | 528 | 396 | 76 | 2.13 | 504 | 99 | 1.6 |
| UPC-3 | 59 | 27 | 1.16 | 2.60 | 573 | 369 | 59 | 3.14 | 400 | 82 | 1.1 |
| Min | 15 | 18 | 0.85 | 0.85 | 439 | 287 | 59 | 1.51 | 68 | 26 | 1.1 |
| Max | 288 | 32 | 1.27 | 2.92 | 600 | 481 | 152 | 4.04 | 1104 | 333 | 9.1 |
| Mean | 134 | 26 | 1.06 | 2.00 | 517 | 390 | 93 | 2.37 | 428 | 128 | 5.0 |
| SD | 94 | 5 | 0.13 | 0.62 | 55 | 53 | 25 | 0.68 | 256 | 83 | 3.1 |

During the initial screening, infiltration rate showed moderate to strong correlations ($|r| > 0.30$) with several variables. Infiltration was negatively correlated with silt content, bulk density, penetration resistance and epigeic earthworm biomass, and positively correlated with sand content, soil moisture, anecic biomass and total earthworm biomass. Following collinearity checks and variable selection, bulk density, sand content and total earthworm biomass were retained as predictors for the linear model. Soil moisture was initially included among candidate predictors but was not retained in the final model due to its lack of statistical significance and a slight decrease in model performance.

The linear model including bulk density, sand content and total earthworm biomass explained a substantial proportion of the variability in infiltration rate ($F = 11.23$, $df = 3, 11$; $p = 0.001$; adjusted $R^2 = 0.69$). All predictors were significant: bulk density was negatively related with infiltration rate, whereas sand content and earthworm biomass showed positive associations (Table 2).

Table 2. Results of the linear model explaining variation in infiltration rates across the study sites. Reported values include model estimates, standard errors, t values and associated p values for each covariate included in the final model.

| Covariate | Estimate | Std. Error | t value | Pr(> t) |
|-------------------|-----------------|-------------------|----------------|---------------------|
| (Intercept) | 134.470 | 13.640 | 9.855 | < 0.001 |
| BD | -55.610 | 14.240 | -3.907 | 0.002 |
| Sand | 51.190 | 14.300 | 3.581 | 0.004 |
| Earthworm biomass | 37.470 | 14.380 | 2.607 | 0.024 |

5.4 Discussion

This chapter addresses water infiltration in urban environments by analysing infiltration rates across a range of green spaces and examining how infiltration varies within the urban context. It also discusses the role of physical and biological soil properties in controlling water infiltration.

To date, assessment of soil water infiltration in urban green spaces remain overall limited. With regard to the city of Milan, to our knowledge the only available study is that by Galli et al. (2021), which focused on small urban green elements (such as green sidewalks and roundabouts) characterised by degraded conditions or recent restoration, and assessed soil hydraulic behaviour through measurements of unsaturated hydraulic conductivity. In contrast, the present study quantifies steady-state surface water infiltration and extends the assessment of urban soil infiltration to a broader range of green spaces, encompassing sites differing in size, soil properties, vegetation type and disturbance levels.

Across the investigated green spaces, infiltration rates varied widely, ranging from 15 to 288 mm h⁻¹. Such wide variability is a well-documented feature of urban soils, with even broader ranges reported in the

literature (e.g. 1–679 mm h⁻¹, Yang and Zhang, 2011; 2–255 mm h⁻¹, Wang et al., 2018; 20–1870 mm h⁻¹, Paradelo et al., 2025). According to FAO classification criteria, the infiltration rates observed in this study can be considered moderate to high. This is consistent with findings from other urban contexts, where urban green soils have been shown to maintain good infiltration capacity (Whitehead et al., 2021; Paradelo et al., 2025).

However, to assess the functional relevance of the observed infiltration rates, these values need to be interpreted in relation to local precipitation regimes. Using precipitation records from the ClimaMi project (2012–2020, www.progettoclimami.it), based on three meteorological stations spanning the northern, central and southern sectors of the city, we contextualised the observed infiltration rates within rainfall conditions experienced in Milan, considering both typical rainfall intensities and maximum intensities recorded over time. Rainfall intensities in the dataset were recorded at a 10-min temporal resolution. When compared with mean rainfall intensities over the 2012–2020 period, summarised at seasonal scales (Table 3), infiltration rates in all sites greatly exceeded typical precipitation inputs throughout the year, indicating that the investigated soils could be able to infiltrate rainfall conditions most commonly experienced in the city.

Table 3. Seasonal rainfall intensity recorded in Milan over the 2012–2020 period from the ClimaMi project (www.progettoclimami.it). Values represent the average of mean rainfall intensity across three meteorological stations and the highest maximum rainfall intensity recorded among the stations. Rainfall intensities were recorded at a 10-min temporal resolution.

| Season | Mean rainfall intensity (average of 3 stations, mm h ⁻¹) | Max rainfall intensity (highest value across stations, mm h ⁻¹) |
|--------|--|---|
| Winter | 0.8 | 87.0 |
| Spring | 1.8 | 96.7 |
| Summer | 3.8 | 92.9 |
| Autumn | 2.1 | 86.3 |

Note. Meteorological stations:

Milano-Bicocca: 45.51017° N, 9.21158° E

Milano-Centro: 45.45964° N, 9.19491° E

Milano-Sud: 45.43129° N, 9.20050° E

A different picture emerges when considering maximum rainfall intensities recorded. Seasonal maxima ranged from 86 mm h⁻¹ recorded in autumn to 97 mm h⁻¹ in spring (2012-2020). Only about half of the study sites exhibited infiltration rates exceeding these values, suggesting that a substantial fraction of soils in Milan’s green spaces may be unable to infiltrate rainfall occurring at very high intensities.

Moreover, it should be noted that field infiltration measurements represent potential infiltration rates under controlled conditions, in which water is applied gradually and surface disturbance is minimised. Under natural rainfall, raindrop impact and the resulting surface sealing may reduce effective infiltration, with these effects

being amplified under high intensity events (Hillel, 1998). As a result, infiltration rates measured in the field may overestimate effective infiltration under real rainfall conditions.

Attempts to quantify this overestimation have yielded variable results, and no univocal estimate has emerged (Auteri et al., 2020). Reported differences depend on several factors, including soil texture, structural stability, initial moisture conditions and vegetation cover (Di Prima et al., 2017). Comparative studies indicate overestimation factors ranging from values of about 2–3 under simulated rainfall intensities of 100–200 mm h⁻¹ (Bhardwaj and Singh, 1992) to substantially higher values (up to about eightfold; e.g., Cerdà, 1996).

Considering the rainfall intensities examined in this study and the predominantly vegetated conditions of the sites, an overestimation factor on the order of two is used here as a plausible reference for interpreting field-measured infiltration rates. Under this assumption, only about one third of the investigated sites would be able to effectively infiltrate rainfall occurring at the highest recorded intensities. These sites were not confined to a single green area category, but occurred across different typologies, including peri-urban parks (both grassland and forests; PP and PPF) and urban parks (UP).

However, the extent to which infiltration may be effectively limited under high intensity rainfall strongly depends on vegetation cover (Hillel, 1998), which is not uniform across sites. In forested sites, canopy interception and the presence of litter layer reduce both the amount of rainfall reaching the soil surface and the kinetic energy of raindrop impact; under these conditions, infiltration limitations are unlikely, even under the highest rainfall intensities. A similar buffering effect can be expected in grassland sites where mowing frequency is low and grass is allowed to grow taller, as commonly occurs in peri-urban parks managed under low-intensity regimes and, to some extent, in some urban parks. By contrast, grasslands in central urban parks and small urban green islands are typically subject to frequent mowing and intensive trampling, resulting in shorter and sometimes discontinuous vegetation cover. In this context UPC, characterised by lower infiltration rates and limited vegetation buffering, emerged as the most vulnerable green spaces with respect to high-intensity rainfall events.

Focusing on field-measured infiltration rates, the absence of significant differences among green area categories suggests that infiltration in Milan's urban soils is primarily controlled by site-specific soil conditions rather than by land use or vegetation type (grassland and forest). Linear model results identified soil compaction (as reflected by bulk density), sand content and earthworm biomass as the main predictors of infiltration rates across the study sites.

Bulk density was negatively associated with infiltration rates in the linear model, in line with previous studies highlighting the central role of bulk density in controlling spatial variability of infiltration in urban soils (Yang and Zhang, 2011). In fact, in our dataset, in the exploratory analysis infiltration was positively correlated with distance from the city centre, reflecting the urban–peri-urban gradient in soil compaction documented in the

previous chapters. However, as bulk density values across the study sites remained below commonly reported critical thresholds (e.g. $< 1.6 \text{ g cm}^{-3}$; USDA, 2023), infiltration was never severely restricted, even in the relatively more compacted sites.

Sand content was positively associated with infiltration rate, consistent with the well-known influence of soil texture on water movement. All investigated soils were characterized by loam or sandy loam textures, which are generally favourable to infiltration (Hillel, 1998).

Earthworm biomass emerged as one of the main predictors positively influencing water infiltration. This result is well supported by a substantial body of literature, particularly from agricultural contexts (Lamandé et al., 2003), whereas evidence from urban ecosystems is lacking.

In this study, anecic earthworms accounted for approximately 70% of total earthworm biomass. Anecic are widely recognized as the primary drivers in regulating soil infiltration processes, due to their large body size and their burrowing behaviour. They construct networks of deep, vertical burrows that open directly to the soil surface and can persist over time (Shipitalo and Le Bayon, 2004; Hoeffner et al., 2022). Although anecic biomass was positively related to infiltration in the exploratory correlations, total earthworm biomass provided a better fit in the multivariate model, suggesting an additional, non-negligible contribution from endogeic earthworms. Epigeic species may also influence water infiltration; however, their effect is confined to the very first centimetres of soil and, as a result, their overall contribution to soil water movement is limited and has received little attention. Given also their marginal representation in the present study, a substantial contribution of epigeic species to the observed infiltration patterns can reasonably be excluded.

Endogeic earthworms have received less attention in infiltration studies, as their burrows are typically smaller, often discontinuous, lack a preferential orientation, and are not directly connected to the soil surface (Shipitalo and Le Bayon, 2004). Consequently, the role of endogeic earthworms in soil hydrology has traditionally been associated mainly with soil water retention and storage. However, as shown for example by Capowiez et al. (2021) in controlled conditions, when burrowing activity is high, endogeics can also contribute to enhancing water infiltration, although to a lesser extent than anecic earthworms.

Finally, a limitation of this study should be acknowledged. Infiltration was assessed at a limited number of sites and based on a single measurement per site, without repetition. This may have contributed to the high variability observed in infiltration rates and may have reduced the ability to detect differences among green area categories.

5.5 Conclusions

Overall, our results suggest that soils in Milan's green spaces are generally able to infiltrate typical rainfall commonly experienced in the city, whereas the highest intensities recorded over time may exceed infiltration capacity, in line with findings from other urban contexts (Wang et al., 2018; Ungaro et al., 2022). This issue is particularly relevant in light of climate change, which is increasing the frequency and magnitude of extreme rainfall events in the Alpine–Mediterranean region (Brunetti et al., 2004).

Infiltration rates showed high spatial variability across the urban context, but no significant differences were detected among green area categories. This suggests that, in Milan, infiltration is primarily governed by site-specific soil conditions rather than by land use or vegetation type per se. Soil compaction, sand content and earthworm biomass emerged as the main drivers of infiltration, highlighting the joint control exerted by physical and biological soil properties. While the individual influence of these factors is well documented, the present results emphasise that the maintenance of infiltration depends on the combined contribution of relatively stable soil attributes (such as texture), dynamic physical conditions (such as compaction) and soil biota.

Our results support the view that earthworms, especially anecic species, are active contributors to infiltration also in urban environments. This finding is particularly noteworthy given that, while their role in promoting infiltration is well documented in agricultural systems, evidence from urban soils remains limited.

These findings suggest that maintaining or enhancing infiltration capacity in urban green spaces requires integrated management approaches that address both soil physical condition and biological functioning. Practices aimed at limiting mechanical compaction, increasing organic matter inputs and preserving surface protection—such as avoiding bare soil, maintaining litter layers or dense herbaceous cover—are likely to play a central role. By simultaneously reducing compaction risk and promoting favourable conditions for soil fauna, such measures can contribute to sustaining water infiltration and, more broadly, to the effectiveness of urban green infrastructure in stormwater management.

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Supplementary Materials

ANOVA RESULTS FOR INFILTRATION RATES ACROSS GREEN AREA CATEGORIES

Notes: *Inf_rate*: infiltration rates. *PPf*: peri-urban forest, *PP*: peri-urban park, *UP*: urban park, *UGI*: urban green island, *UPC*: urban park centre.

Model: *Inf_rate* ~ *Green_area*

Residuals:

| Min | 1Q | Median | 3Q | Max |
|---------|--------|--------|-------|--------|
| -118.67 | -42.33 | 1.00 | 43.67 | 151.33 |

Coefficients:

| | Estimate | Std. Error | t value | Pr(> t) |
|----------------------|----------|------------|---------|----------|
| (Intercept) | 133.67 | 50.78 | 2.632 | 0.0251 * |
| <i>Green_areaPPf</i> | 36.00 | 71.82 | 0.501 | 0.6270 |
| <i>Green_areaUGI</i> | -13.67 | 71.82 | -0.190 | 0.8529 |
| <i>Green_areaUP</i> | 75.00 | 71.82 | 1.044 | 0.3209 |
| <i>Green_areaUPC</i> | -93.33 | 71.82 | -1.300 | 0.2229 |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 87.96 on 10 degrees of freedom

Multiple R-squared: 0.3801, Adjusted R-squared: 0.1322

F-statistic: 1.533 on 4 and 10 DF, p-value: 0.2654

CHAPTER 6

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

6.1 General conclusions

This thesis investigated urban soils within public green spaces of the city of Milan, Italy, with the objective of evaluating the ecological condition and functioning of Milan's urban soils, through the integrated analysis of multiple soil dimensions. In doing so, the thesis also examined whether scientific evidence supports the common perception that urban soils are affected by widespread ecological degradation.

This thesis focused on a well-defined subset of urban soils, namely those occurring in publicly accessible green spaces designed for recreational and ornamental purposes, including urban parks, neighbourhood green areas, and small vegetated patches such as traffic islands. These spaces represent an essential component of urban environments, as they constitute some of the few remaining areas where unsealed soils and associated ecological processes may persist within densely built cities (Morel et al., 2015). At the same time, their soils are directly exposed to human activities and urban pressures, making them particularly suitable for investigating the ecological effects of urbanisation.

The assessment of soil physical and chemical properties indicated that the investigated soils generally exhibit favourable conditions for soil ecological functioning. Most soils were characterised by high SOC contents and pH values within ecologically favourable ranges, and no evidence of problematic compaction was observed. Overall, these results suggest that the soils of the investigated areas were not excessively altered by anthropogenic activities. Nevertheless, urban pressure left a clear imprint on several parameters, which consistently reflected increasing urban pressure along an urban–peri-urban gradient. Peri-urban parks—both grassland (PP) and forested areas (PPf)—retained more semi-natural soil characteristics, whereas urban areas—particularly urban parks in the city centre (UPC) and urban green islands (UGI)—showed stronger signs of alteration. These patterns reflect differences in management intensity, recreational pressure and land-use history, and were more pronounced in the topsoil (0-10 cm), where the effects of human activities are strongest.

Earthworm communities confirm the overall favourable ecological condition of the investigated soils. High values of abundance, biomass, and taxonomic richness, compared to global values observed in urban context, indicate the presence of viable earthworm communities that do not show evidence of a generalized ecological degradation. At the same time, clear responses to urban pressures were detected. In particular, Pb content emerged as one of the main drivers, with consistent negative effects across multiple attributes of earthworm communities, influencing biomass, taxonomic richness, and functional structure. Earthworm ecological categories exhibited differentiated responses to increasing Pb levels, with anecic earthworms showing greater sensitivity, while endogeic species appeared less affected. Urban pressures also had a clear effect on shaping the functional composition of earthworm communities, which differed between urban and peri-urban green spaces, consistently with contrasts in management intensity, human use, land-use history, and associated soil

alterations. Endogeic earthworms, more tolerant to disturbance, predominated in urban contexts, whereas anecic earthworms, more ecologically demanding, were more abundant in peri-urban parks, particularly in forested areas. Epigeic earthworms, although rare overall, occurred mainly in peri-urban parks.

In contrast to earthworm communities, the application of the QBS-ar index depicted a generally modest level of biological soil quality across the investigated sites, with only about one third exceeding the thresholds for good biological quality proposed in the literature (Menta et al., 2018). QBS-ar values were nonetheless broadly consistent with those reported for other Italian urban contexts, although slightly lower overall (Ungaro et al., 2022). Peri-urban forests emerged as important habitats for disturbance-sensitive microarthropods, confirming their central role in maintaining soil biodiversity in urban environments, whereas central urban parks exhibited the lowest QBS-ar values, in line with historically higher levels of anthropogenic pressure. While capturing these broad ecological contrasts, the index was only weakly responsive to fine-scale differences in management, disturbance intensity, or soil properties. As a consequence, its effectiveness in capturing differentiated urban pressure effects was reduced. Nevertheless, QBS-ar proved to be a simple and synthetic tool to characterise soil microarthropod communities in urban green spaces and providing a general assessment of soil biological quality.

As discussed above, the two faunal components analysed, earthworms and microarthropods (QBS-ar), provided partially contrasting indications of the ecological status of Milan's urban soils. This divergence could reflect different responses of the two groups to urbanisation; however, based on the results obtained, no clear evidence emerges in support of this interpretation. An alternative explanation is that the observed discrepancy is related to the marked seasonal variability of microarthropod communities, which makes QBS-ar values strongly dependent on the specific sampling period (e.g. autumn or spring) (Aspetti et al., 2010). The application of the index in a single season may therefore have resulted in a partial assessment of soil biological status. In any case, the contrasting evidence from earthworms and microarthropods highlights the importance of integrating multiple faunal components to achieve a more robust and reliable assessment of the ecological condition of urban soils, especially when these fauna groups cover different functions.

In urban environments, soil fauna responses may diverge from patterns observed in soil properties and urban pressure due to site specific factors, such as land-use legacies and recent human interventions (Joimel et al., 2017). In our study, this was observed in urban green islands (UGI). Despite their small size, isolation, and intensive management, and despite soil chemical and physical properties reflecting anthropogenic disturbance, some UGI exhibited unexpectedly high QBS-ar values and earthworm abundance and biomass. This pattern is probably related to recent green space restyling interventions, which involved soil replacement with material originating from less disturbed environments and may have introduced already rich and structured faunal communities, that persisted despite disturbance. This finding emphasises the heterogeneity of urban soils and the role of site-specific histories in shaping soil fauna patterns.

Beyond soil properties and biotic communities, soil functioning was also explored in terms of water infiltration capacity. When considered in relation to local precipitation regimes, infiltration rates indicated that soils in Milan's green spaces are generally able to infiltrate typical rainfall events, whereas the highest rainfall intensities recorded over time may exceed infiltration capacity. Urban parks in the city centre (UPC), characterised by lower infiltration rates and limited vegetation buffering, emerged as the most vulnerable green spaces with respect to high-intensity rainfall events. No significant differences in infiltration rates were detected among green area categories, suggesting that, in Milan, infiltration is primarily governed by site-specific soil conditions. Infiltration rates were influenced by the combined contribution of soil physical and biological properties, with bulk density, sand content and earthworm biomass emerging as the main drivers. Even in the absence of clear differences among green area categories, spatial variability in infiltration rates—being partly controlled by soil compaction—reflected the urban–peri-urban gradient in bulk density, with infiltration rates generally increasing away from the city centre.

As outlined in the General Introduction, soil compaction and its potential implications for soil ecological functioning originally constituted the central research question of this thesis. This focus was subsequently reframed, as soil compaction across the study area did not reach critical thresholds. Nevertheless, compaction-related metrics (bulk density and penetration resistance) proved to be sensitive indicators of varying levels of anthropogenic pressure and exhibited a transversal role across multiple soil dimensions. In particular, they contributed to modulating both soil biotic responses and water infiltration capacity. Accordingly, soil compaction emerged not as a relevant cause of soil degradation, but as a key factor shaping the functioning of the investigated urban soils.

Overall, this thesis provides an integrated overview of the ecological condition and functioning of soils in public green spaces in Milan. Its main strength lies in the adoption of a multi-dimensional approach—still relatively uncommon in urban soil studies—based on the joint analysis of different components of the soil ecosystem and their interactions in shaping soil functions. This work contributed to improving the understanding of how urban pressures influence soil characteristics and to the broader scientific discussion on urban soil functioning. At the same time, it helped to fill a substantial knowledge gap regarding the soils of Milan, which have remained largely unexplored, and provided the first dataset on earthworm communities in urban environments in Italy.

The results showed that, despite the anthropogenic pressures to which they are exposed, soils in public green spaces generally exhibit favourable ecological conditions and are able to sustain multiple ecosystem services. This highlights the functional relevance of urban green space soils for maintaining environmental quality within cities. The analysis of soil physical and chemical properties showed that most soils were characterised by relatively high organic matter content, pH values suitable for biological activity, and low levels of compaction. Under these conditions, Milan's green spaces are generally able to sustain soil biodiversity.

Indeed, soil fauna displayed overall good ecological status, particularly earthworm communities, while microarthropod communities indicated a more moderate biological quality. Given the role of earthworms and microarthropods in key soil processes, including soil structure modification, water regulation, and nutrient cycling through detrital food webs (Lavelle et al., 2006; Blouin et al., 2013), well-developed soil faunal communities contribute to the maintenance and enhancement of soil functioning in Milan's public green spaces. Finally, water infiltration rates in public green space soils, positively influenced by soil physical properties and high earthworm biomass, were generally consistent with the rainfall intensities most commonly experienced in the city. Therefore, soils in Milan's public green spaces contribute to the overall permeability of the city.

Despite the generally favourable conditions observed, clear and consistent differences between urban and peri-urban contexts emerged across all the soil components considered. These patterns reflect contrasts in management intensity, land-use history, and levels of human use. Accordingly, urban green spaces—particularly parks in the historical city centre (UPC)—showed more pronounced signs of alteration, whereas peri-urban parks retained ecological characteristics typical of less disturbed systems.

To conclude, our findings challenge the assumption of pervasive ecological degradation of urban soils, highlighting the need for a change in perspective. Rather than being viewed merely as inert substrates that can be modified or exploited, urban soils should be recognised as an environmental heritage that must be preserved to maintain its ecological functionality.

6.2 Future perspectives

Future research could expand the number of investigated sites for soil fauna and water infiltration measurements, in order to obtain a more comprehensive picture of urban soil ecological conditions and strengthen the robustness of the observed patterns. With specific regard to soil microarthropods, repeating QBS-ar assessments across different seasons would allow a more complete evaluation of soil biological status, while also supporting the assessment of the index in a monitoring perspective. The limited ability of QBS-ar to discriminate subtle ecological differences among sites may be partly related to the fact that the index is based exclusively on the presence of taxonomic groups and their degree of adaptation to the edaphic environment, without also accounting for their abundance. In this context, the application of QBS-ab (Mantoni et al., 2021), an abundance-based modification of the QBS-ar index, could be explored to evaluate whether the inclusion of abundance enhances sensitivity to finer-scale differences in management, disturbance intensity, and soil properties. Finally, the management insights emerging from this work could be further developed and integrated, contributing to a more informed approach to soil monitoring and management in urban green spaces.

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