Urban Ecosystems (2026) 29:42
https://doi.org/10.1007/s11252-026-01908-6

RESEARCH

The potential use of behavioural bioassays as a first-tier approach for screening urban soil biodiversity: a pilot study

Lorenzo Federico[1] · Valeria Tatangelo[1] · Francesca Pittino[1] · Claudia Russo[1] · Emanuele Vegini[1] · Sandra Citterio[1] · Andrea Franzetti[1] · Sara Villa[1]

Received: 3 November 2025 / Accepted: 13 January 2026

Abstract
Soil health represents a key component of urban ecosystems and a priority for achieving the European Union's climate neutrality and biodiversity restoration goals. Changes in edaphic diversity are the first indicators of soil health, but require long investigation times. Therefore, rapid, multi-level, and low-impact diagnostic tools are required. Behavioural bioassays, including avoidance and disaggregation tests, serve as rapid and ecologically relevant indicators for identifying soils subject to population decline. However, the metrics of in situ biodiversity loss and the laboratory-based ecotoxicological responses are not aligned. This pilot study investigates the potential use of the behavioural endpoints as screening indicators of biodiversity in invertebrate and bacterial communities in three urban soils. Multi-species bioassays were employed using model organisms with contrasting morpho-ecological traits, i.e. soft-body (earthworms) and hard-body (collembolans, and terrestrial isopods), to evaluate soil quality gradients. The behavioural results were then compared with ecological biodiversity data concerning the soil fauna and microbial communities. The behavioural responses of model organisms consistently aligned with reductions in invertebrate biodiversity, indicating habitat population decline. These changes, however, did not emerge from microbial analysis, suggesting that links between organismal responses and microbial diversity are yet to be investigated. The results support the use of behavioural bioassays, in combination with faunal diversity assessments, as an effective first-tier screening tool for urban soil health evaluation. This multi-level framework enhances the resolution and efficiency of soil quality monitoring and supports targeted management interventions in degraded urban environments, as well as in peri-urban, agricultural, and other human-impacted landscapes.

Keywords Behavioural test · Soil biodiversity · Porcellionides pruinosus · Folsomia candida · Eisenia fetida · Urban ecosystems

Introduction

Recent years have seen a heightened focus on monitoring and remediating soil ecosystems, driven by the need to implement effective environmental strategies for ecological transition and to counteract climate change effects (Bowler et al. 2010). A significant development in this context is the adoption by the European Union of legislation to make soil health monitoring mandatory, including the proposed directive on Soil Monitoring and Resilience presented by the European Commission (EC 2025a). This directive establishes guiding principles for sustainable soil management and addresses situations where soil stressors pose unacceptable health and environmental risks. Since more than 60% of European soils are depleted, monitoring and prioritisation strategies are required to assess and achieve the agreed EU climate and biodiversity goals. Concurrently, the global community has recognised the urgency of soil ecosystem restoration, culminating in the establishment of the UN Decade on Ecosystem Restoration, which aims to encourage a coordinated and comprehensive approach to the restoration of degraded ecosystems and stem the rapid decline of biodiversity (UNEP and FAO 2020). Among the main stressors, pollution has a significant impact on soil health (Vieira

✉ Lorenzo Federico
lorenzo.federico@unimib.it

[1] DISAT, Department of Earth and Environmental Sciences, University of Milano- Bicocca, Piazza della Scienza 20126, Milan, Italy

Published online: 30 January 2026

et al. 2024), and the development of a framework for the regular assessment of soil pollution is strongly urged by the European Commission, in line with the Zero Pollution Action Plan (EC 2021).

To provide an initial snapshot of soil quality, the study of changes in edaphic communities represents a first relevant approach (Linden et al. 1994; Schloter et al. 2003; Santorufo et al. 2012). Quantifying both invertebrate and microbial biodiversity is necessary to evaluate soil health, and represents the key element of the Nature Restoration Law (EU, 2024), and the Sustainable Development Goals (SDGs) (Sachs 2012). Despite their importance, diversity analyses are primarily diagnostic and demand substantial time and costs. Therefore, it is crucial to implement rapid and cost-effective prognostic strategies to effectively support these studies.

Behavioural bioassays of edaphic organisms represent ecologically relevant indicators of changes in soil conditions (Coyle et al. 2017). Among the behavioural tests, those related to avoidance responses (ISO 2020a, 2020b) and alteration of gregariousness in social edaphic organisms (Federico et al. 2024) can be used as prognostic early-warning tools to detect effects on population decline and/or fragmentation at population level. Since these behavioural traits are indicative of limited habitat function, soils that induce such responses are expected to support reduced biodiversity, as more sensitive and vulnerable species will avoid threatened conditions. Most soil taxa exhibit limited long-distance mobility and are therefore unable to migrate across broader spatial scales (Coyle et al. 2017). Avoidance responses are expressed as active displacement away from stressed soils (Gainer et al. 2022), resulting in reduced residence time and constrained colonisation of affected patches. In contrast, disaggregation reflects a fragmentation of the gregarious behaviour of isopods, which may occur both within stressed soils and in adjacent buffer zones, where individuals redistribute locally rather than abandoning the area entirely (Federico et al. 2024). It follows that over time, such responses can result in local redistribution, population decline, and loss from the community under continued environmental stress, even in the absence of large-scale dispersal. Consequently, soils that elicit pronounced avoidance or altered social behaviours are likely to exhibit reduced local diversity, not only through migration but also through decreased activity, limited population establishment, and gradual species loss. As recently highlighted by the European Commission (EC 2025b), metrics of biodiversity loss remain poorly aligned with laboratory-based ecotoxicological endpoints. Addressing this gap, the present study moves beyond a priori assumptions by empirically linking behavioural responses to the diversity of invertebrate and bacterial communities in urban soils. The ultimate goal is to contribute to the identification of a tiered and targeted approach, balancing the speed and cost-efficiency of ecotoxicological bioassay with the complexity of biodiversity analysis, in order to guide decision-making in prioritising areas for further investigation.

Materials and methods

Study area and soil properties

Three distinct urban areas under greening strategies (designated as U2, U3, and U4) situated at the Milano Bicocca University campus (Italy, latitude: 45° 30‘49.0497’; longitude: 9° 12‘40.9114’) were considered as a case study (Fig. 1). A comprehensive description of the university square can be found in Picot (2004). Briefly, these patches boast identical extension (15 × 30 m) and share comparable vegetation species but U2 garden is distinguished by its shaded status due to its proximity to university buildings, and such distinct solar irradiance has been resulted in varied levels of student presence, since U3 and U4 areas have been noted to experience a higher influx of students compared to U2, which area remained inaccessible since the 2022 year. Soil sampling activity was conducted in April 2024. Within each green area, a 5 × 10 m grid was established to delineate different plots. Three plots (A, B, and C), representing distinct spatial zones (one central and two peripheral), were selected. Within each selected plot, six replicate samples of approximately 1 kg (10 × 10 × 10 cm) were randomly collected, excluding litter, resulting in a total of 18 samples per green area. Three replicates of each plot were designated for biological behavioural assays and physicochemical characterization, while each of the remaining three replicates was split for the assessment of soil pedofauna structural and functional diversity (QBS-ar) and for microbial diversity analyses. Soil samples were collected using a field shovel cleaned with an ethanol–water solution (70:30, v/v) prior to each sampling to prevent cross-contamination and transported to the laboratory in black plastic bags for subsequent analyses. Aliquots of sampled soils were analysed in terms of texture, water holding capacity (WHC), pH, and soil organic matter (SOM) (Supplementary Materials - SM1). These parameters were considered as the main core descriptors of soil health and habitat quality according to the Soil Monitoring and Resilience Directive (EC 2025a). Before each analysis, all samples were allowed to dry under a hood for evaporation of the water content, and then placed in an oven at 105 °C overnight for total drying.

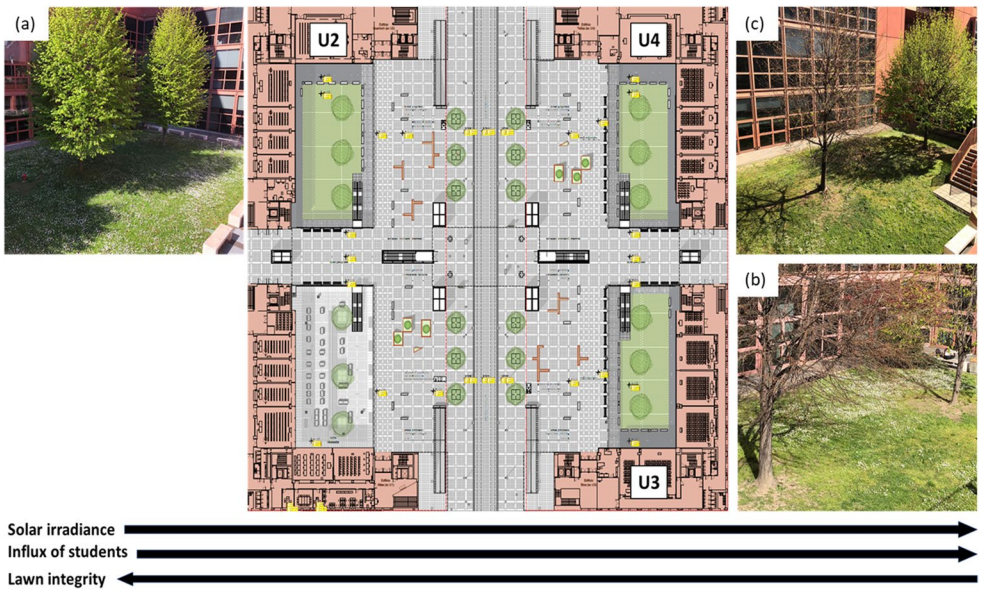

Fig. 1 Map of Piazza della Scienza with the respective green areas, designated as U2 (**a**), U3 (**b**) and U4 (**c**), located within the University of Milan Bicocca campus. The three green areas are characterised by different irradiation gradients, afflux of university community and lawn integrity (black arrows). Source of floor plan: Studio Aegis, Brescia, Italy

Photosynthetic efficiency

In each green area of the campus there are three individuals of *Tilia spp*. on which photosynthetic efficiency parameters were measured during the same sampling period, in order to confirm any differences induced by solar exposure levels. By measuring chlorophyll fluorescence efficiency, it is possible to obtain information on photosynthetic activities, since the intensity of fluorescence emission is inversely proportional to the amount of solar radiation used during photosynthetic processes (Maxwell and Johnson 2000). Leaves were taken from branches in a position of full light, at the top of the canopy, potentially subjected to a maximum photosynthetic activity (Gottardini et al. 2016). For each individual, 15 leaves were collected and chlorophyll a fluorescence was measured with the Handy-PEA fluorometer (Hansatech Instruments, Pentney, Norfolk, UK). Among the parameters measured by the instrument, Fv/Fm is the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) after dark adaptation, representing the maximum quantum yield of photosystem II. This parameter is the most widely used to investigate the photosynthetic aspects of a plant species (Sepúlveda and Johnstone 2018; Callow et al. 2018).

Behavioural bioassays

Individuals of *Porcellionides pruinosus* (Brandt, 1833), *Eisenia fetida* (Savigny, 1826), and *Folsomia candida* (Willem, 1902) were provided from the Laboratory of Ecotoxicology of Milano Bicocca University (Italy) (SM2). All the model organisms were used for performing the avoidance bioassays, following the protocol outlined in ISO guidelines (2020a, 2020b), while only terrestrial isopods were used for investigation of the avoidance and disaggregation effect (Federico et al. 2024). The soils sampled from the green areas (U2, U3 and U4) were compared with standard laboratory soil (LUFA 2.2), in order to evaluate the attractive, elusive or indifferent responses of the tested species to urban soils. Plastic boxes (170 × 120 mm) were used as test arena for earthworms and terrestrial isopods, and Petri dishes (⌀ = 100 mm) for collembolans. In one side of the container, 100, 50, and 10 g d.w. of sample soils per each green area and replicates were added, in the earthworms, terrestrial isopods, and collembola bioassays respectively. On the other side, the same relative quantity of soils was filled using the standard LUFA Speyer 2.2 soil (batch no. SP2.2 2123). The chemical and physical characteristics of this standard soil are reported in SM2. Dual controls were performed using only LUFA 2.2 soil on both sides of the box to infer the homogeneous distribution of the organisms and validate the tests. The moisture related to the experiments of terrestrial isopods, collembolans, and earthworms was maintained at WHC of 40%, 50%, and 60%, respectively. For each experiment, ten individuals of the representative species were added separately in any tests and five replicates were performed for statistical reasons. The tests were carried out in thermostatic chambers at 21 ± 2 °C, photoperiod 16:8 h light: dark for 48 h. During the test, the animals were not fed. After 48 h, plastic boxes were gently removed from the thermostatic chambers, and high-resolution colour pictures were taken and processed for the statistical analysis. Avoidance behaviour was expressed as Avoidance (A%):

$$A = \frac{C - T}{n} \times 100 \quad (1)$$

Where C = number of individuals located in the LUFA reference soil compartment, T = number of individuals located in the sampled soils, and n = the total number of live individuals at the end of the experiment. Positive values indicate avoidance behaviour, whereas negative values indicate attraction to the urban soil. Non-avoidance occurs when the distribution of organisms is approximately equal between treated and control soils. Sample soils with less than 20% of individuals were considered as having "limited habitat functions" (ISO 2020a, 2020b).

The disaggregation effects were measured by the disaggregation index (DI):

$$DI = \frac{s + 2d}{n} \times 100 \quad (2)$$

were s = number of groups with only 1 individual, d = number of groups with only 2 individuals, and n = the number of alive individuals at the end of the experiment. The index varies between 0 and 100, which represent the maximum degree of aggregation and disaggregation, respectively, while 50 was fixed as the threshold level above which disaggregation affects 50% of the population. Further details referred to the index can be found in Federico et al. (2024).

Biodiversity analysis

Before soil arthropods extraction using the Berlese-Tullgren method, any earthworms (Lumbricinae) and snails (Gasteropoda) were removed manually and counted from each soil sample. Soil arthropods extraction was conducted within 48 h of the sampling time using a Berlese-Tullgren extractor, following the methodology outlined in Parisi et al. (2005). The extractor was basically composed by a sieve (mesh of 2 mm, ⌀ = 20 cm), resting on a plastic funnel whose end part is inserted inside a plastic container filled with 2:3 ethanol and 1:3 of glycerol. From the freshly sampled soil samples, 950 g were placed inside the sieve and placed under incandescent lamps (40–60 W) at 30 cm of distance. In this way, the soils gradually dry out and the aridity of the soil forces the fauna present in the sample to avoid towards the depth of the sieve, until they fall and are captured inside the container with the alcohol and glycerol solution. The extraction of edaphic organisms took place for 7 days. The collected organisms were therefore analysed under a stereomicroscope at low magnification and classified at order/class level according to the major taxonomic groups listed in the standard table of Parisi et al. (2005). The remaining 40 g of soil from each replicate was used for the extraction of enchytraeids and nematodes, with 20 g allocated for each extraction. For enchytraeids, soil was mixed with 96% ethanol (1:5 ratio), topped with distillate water, and stained with 10 drops of rose Bengal (Pereira et al., 2018). Nematodes were extracted for 7 days using the tray method (McSorley 2000).

For each sample, the total number of individuals (N), taxa (S), and density (ρ) per volume of soil extracted (N/m³) were assessed. The levels of edaphic biodiversity of the soil meso and macrofauna were quantified using structural synthetic diversity indices (Table 1).

The A/C ratio structural index (Bachelier 1986) was calculated based on the most abundant group of arthropods, Acarina (A) and Collembola (C), respectively. Functional metric relating to the QBS-ar index (acronym of Soil Biological Quality based on arthropods) was calculated through the sum of the ecomorphological indices (EMI) for each arthropod detected on each soil (Parisi et al. 2005). The EMI value ranges from 1 (epigeous species) to 20 (euedaphic species). Some taxonomic groups have a single EMI value because all species within the group exhibit the same level of adaptation to soil, whereas other groups are characterized by a range of EMI values, reflecting different degrees of soil adaptation among species (Menta et al. 2018). The QBS-ar value for each green area was calculated by summing the EMI values assigned to the taxa identified in the extracted samples. When more than one EMI value was attributed to the same taxon, only the highest EMI value was considered in the QBS-ar calculation.

Molecular analysis

The diversity of bacterial communities in the soil can reflect important ecological functions and is closely linked to habitat quality and the availability of resources for soil fauna (Van Elsas et al. 2006; Hermans et al. 2017). For this reason, we investigated whether the characterization

Table 1 List of utilised structural indices, where N is the total number of individuals, S the number of taxa, pi the relative abundances, and H, H_1, and H_2 represent the surrogate hill's numbers for richness, Shannon-Wiener and Simpson indices, respectively

Structural Indices	References	Formula	
Richness indices	Margalef (1958)	$d = \frac{S-1}{lnN}$	(Eq. 3)
Abundance indices	Shannon-Wiener (1948)	$H = \sum_{i=1}^{s} - piln(pi)$	(Eq. 4)
	Simpson (1949)	$D = \sum_{i=1}^{s} (pi)^2$	(Eq. 5)
Absolute Effective Diversity indices (AED)	Gatti et al. (2020)	$AED = H + \frac{H_1^2}{2H_2}$	(Eq. 6)

of the soil bacterial community could reflect the responses provided by behavioural tests. Ten grams of soil from each of the three samples per plot in each green area were pooled together, homogenised, and DNA was extracted from 0.5 g of soil from each pool with the FastDNA™ SPIN Kit for Soil (MP Biomedicals). The characterisation of soil bacterial communities was achieved by sequencing the V5-V6 hypervariable regions of the 16 S rRNA, as outlined by Gandolfi et al. (2024). Amplicon sequence variants (ASVs) were then inferred with a divisive amplicon denoising (DADA2) algorithm (version 1.30.0), as described by Callahan et al. (2016). Forward reads were truncated to 180 bp and reverse reads to 150 bp, Reads containing any ambiguous base calls (Ns) were discarded, reads with an expected number of errors greater than 0.5 were removed for both forward and reverse reads and trimming was done for the first 10 bases of forward reads and the first 20 bases of reverse reads. Classification was done with the Ribosomal Database Project (RDP) 11.4 (http://rdp.cme.msu.edu/).

Data analysis

Statistical analyses were performed with R 4.2.1 (R Core Team 2022). Generalized linear models (GLMs) with a Poisson distribution and a logarithmic link function were used to model the relationship between the independent variable (green areas) and the dependent variables (soil properties, percentage of behavioural alterations in model organisms, individual counts by Order, and number of taxa), accounting for the fact that the three green areas were unreplicated. Analysis of variance (ANOVA) was conducted to assess the effect of the three green areas on Fv/Fm values. Patterns of variation in pedofauna richness of soil across the three green areas were explored using a Principal Component Analysis (PCA) to allow for the reduction of dataset dimensionality while preserving the most significant variance, enabling a refined interpretation of relationships and trends in the biodiversity data. Eigenvalues greater than or equal to 1.0 (Keiser criterion) were considered significant for the extraction of the principal components. To investigate bacterial community diversity, cluster analyses were performed on the Hellinger-transformed ASV table. GLMs with a Poisson distribution corrected for overdispersion were performed on the most abundant classified genera to see their variation according to the area. The ASV table rarefied at 2820 sequences was used to obtain a Venn diagram to investigate the shared ASVs among different areas and to calculate alpha-diversity indices, i.e. Gini index, Shannon index, and the number of ASVs, Chao index was calculated on a non-rarefied dataset (Gini 1912; Shannon 1948; Chao 1984). Differences in the alpha diversity indices according to the sampling areas were further investigated with GLM with a Gaussian distribution. The data were considered statistically significant for values of $p < 0.05$.

Results

Green areas characterizations

Properties of sampled soils (Table SM3) were evaluated to contextualise any differences in environmental conditions that might have influenced behavioural responses or species composition. The three soils displayed the same texture. The soil pH values of the three green areas were near-neutral, ranging from 6.6 to 7.1. On the contrary, U4 showed the lowest level of WHC percentage compared to the soils from U2 ($p < 0.05$), and U3 ($p < 0.001$), the last of which showed the highest WHC_{max} level detected. The SOM % content was significantly higher in U3 soils compared to U2 ($p < 0.01$), and U4 ($p < 0.001$) soils.

Regarding the photosynthetic efficiency in the three areas, the results revealed a statistically significant difference between the groups ($p = 0.016$), and the comparison showed that the Fv/Fm value of U3 was significantly higher than that of U2 (< 0.05, Tukey *post hoc* test). No significant differences were found between U4 and the other groups.

Behavioural bioassays

Within each green area, the three plots (A, B, and C) and their replicates exhibited minimal variability in avoidance responses, with a standard error (SE) lower than 12, 13, and 14% for woodlice, springtails and earthworms, respectively.

Referring to the green areas (U2, U3, and U4), distinct responses emerged (SM4). The avoidance results suggested a no-choice response for all the model organisms tested exposed to U2 soils (Fig. 3a and b, and 3c), and the disaggregation bioassay showed that more than 70% of the terrestrial isopods were aggregated in groups in the condition with U2 soil (Fig. 3d). These results suggest a good quality for the U2 soils in terms of habitat function.

U3 soils elicited attraction in more than 40% of collembolans ($p < 0.05$), and over 70% of terrestrial isopods ($p < 0.0001$) compared to the LUFA soil controls (Figs. 2c and 3b), whereas earthworms displayed no statistically significant preference, despite more than 40% of the individuals being found in U3 soils. Attraction phenomenon could be induced by specific soil properties, but can even be attributed to immobilization or locomotor alterations (Oliveira et al. 2015). In contrast to this hypothesis, the outcomes of *P. pruinosus* showed a higher degree of aggregation (Fig. 3d), suggesting an active behaviour

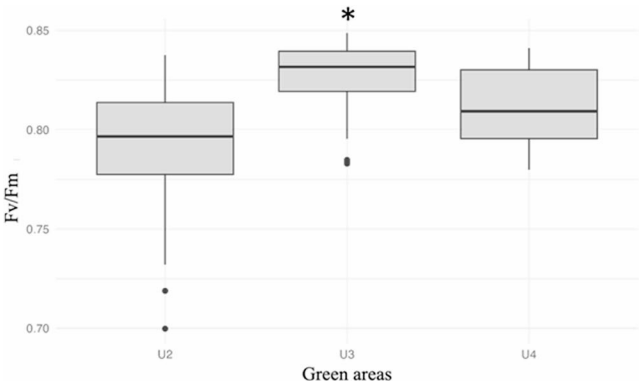

Fig. 2 Box charts referred to the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) of the *Tilia spp*. leaves from U2, U3, and U4 green areas. Data are expressed as mean (± standard error) ($n = 5$ replicates per site). Asterisks denote significant differences to control (Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05)

instead of a lack of locomotion, suggesting that the observed attraction is driven by soil characteristics, such as high organic matter content identified in soil analyses.

In contrast, U4 soils significantly induced a strong avoidance behaviour (Fig. 3) in more than 50% of earthworms ($p < 0.05$), more than 60% of springtails ($p < 0.0001$), and more than 80% of terrestrial isopods ($p < 0.0001$). U4 soils also triggered a strong disaggregation effect on the terrestrial isopod population, showing a disaggregation index (DI) mean of 84% ± 5.2% SE ($p < 0.0001$) (Fig. 3d). The behavioural tests were conducted under controlled laboratory conditions, so it is likely that the limited habitat function observed in U4 soils is due to specific characteristics of these soils. Federico et al. (2024) demonstrated that soil contaminants can induce infochemical disruption; similarly, the effects observed in U4 soils may reflect contaminant presence.

In essence, avoidance responses are ranked in sensitivity as *P. pruinosus* > *F. candida* > *E. fetida*, underlying a different degree of susceptibility of these model organisms. The heightened sensitivity of terrestrial isopods aligns with literature reports of soil contaminant impacts (Maria et al. 2024). Furthermore, the employment of disaggregation indices has enhanced comprehension of soil quality in the case of attractive responses, as emerged from U3 soils. Therefore, the combination of the two ecological endpoints in a single bioassay are a promising tool in the framework of the environmental safety assessment.

Edaphic invertebrates diversity

A total of 1,395 individuals, representing 20 distinct taxa, were extracted from soil collected from the university green areas, showing significant differences in richness and providing valuable insights into the sub-optimal quality of green

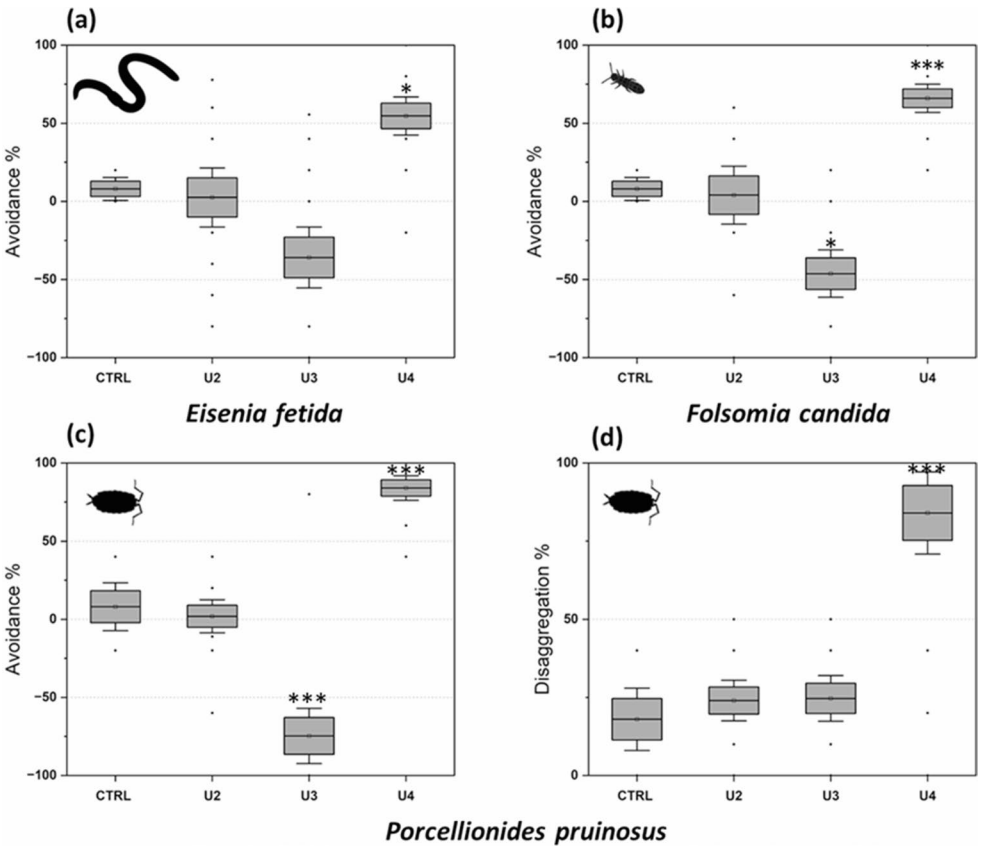

Fig. 3 Box charts referred to the Avoidance percentage (A %) responses in *E. fetida* (**a**), *F. candida* (**b**), and *P. pruinosus* (**c**) populations, with the relative disaggregation index percentage (**d**), exposed to U2, U3, and U4 soils. Data are expressed as mean (± standard error) ($n = 5$ replicates per site). Grey dot lines present the threshold level of ± 50%. Asterisks denote significant differences to control (Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05)

spaces (Table SM5). Almost all taxa, except Symphyla, were found in U2, while the taxonomic richness in U3 and U4 was significantly reduced compared to U2 ($p<0.0001$). GLMs results showed variable responses across the three urban areas. The abundance of Acarina ($p<0.0001$) and Isopoda ($p<0.0001$) was significantly lower in both U3 and U4 compared to U2. The number of Hymenoptera ($p<0.0001$) and Enchytreideae ($p<0.001$) was significantly different only between U2 and U4. Additionally, Diplura ($p<0.0001$) and Pauropoda ($p<0.05$) showed significant differences between U2 and U3. The PCA identified five principal components (PCs), which together explained 74.13% of the total variance in the multifactorial analysis (Table SM6), while 49.8% was explained by the first two components (PC1 and PC2) (Fig. 4). The PC1 accounted for 33.9% of total variance with positive weak loading of Acarina (0.35), Chilopoda (0.36), Protura (0.37) and Isopoda (0.36). In contrast, PC2 accounted for 15.9% of total variance, with positive weak loading of Collembola (0.38), Lumbricidae (0.34), Diplura (0.35), and Symphila (0.32), while Hymenoptera (−0.43) and Gasteropoda (−0.3) showed negative weak loading.

These results emphasise a clear separation of the three urban soil communities, in line with the prediction of previously conducted behavioural tests. Specifically, U2 soils, which did not show restricted habitat functionality, displayed a greater edaphic composition, with a high percentage of species sensitive to changes in soil conditions such as Acarina (32.3%), Protura (7.8%), detected exclusively in this green area, and Isopoda (6.7%) (Toth et al. 2023). Regarding U3 soils, behavioural tests had previously indicated an attraction due to an enrichment

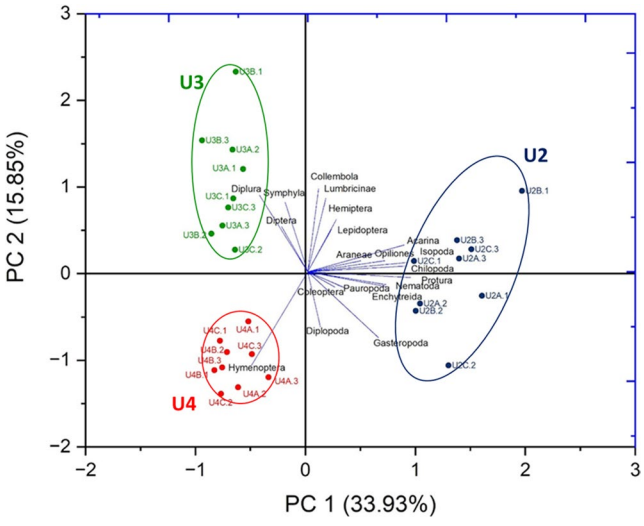

Fig. 4 Principal Component Analysis (PCA) related to the number of individuals per each taxon identified per each urban soil area ($n=18$ replicates per green area), specifically U2 (blue dots), U3 (green dots), and U4 (red dots)

of organic matter, and these results were subsequently confirmed by both the analysis of soil characteristics and diversity analyses. These analyses revealed a community dominated by species sensitive to moisture loss and organic matter content (Lapied et al. 2009), including Collembola (43.1%), Diplura (14.6%), Lumbricinae (4.1%) and Symphila (0.8%), the latter occurring only in U3 green areas. Finally, the results of the behavioural tests in U4 soils outlined a limited habitat function, consistent with the low edaphic composition and with the community partitioning identified through PCA based on taxa abundances. Importantly, the PCA also reflected the reduced contribution of epiedaphic taxa, indicating an overall impoverishment of soil biodiversity, an aspect that would not be captured by indices such as QBS-ar focusing exclusively on edaphic adaptations.

Bacterial communities' diversity

Following high-throughput sequencing, a total of 6,838 ASVs were detected per green area (Figure SM7). The taxonomic composition of the bacterial communities across the three urban green spaces indicated that the most abundant classified genera overall in ascending order were: *Microlunatus* sp. (3.88%), *Nocardioides* sp. (2.26%), *Gaiella* sp. (1.72%), *Agromyces* sp. (2.52%), *Mycobacterium* sp. (0.84%), *Microvirga* sp (0.63%), *Flavobacterium* sp. (0.6%) and *Pedomicrobium* sp. (0.62%). In particular, *Gaiella* sp. ($F_{2,6}$ = 22.50, P_{FDR} = 0.035), and *Pedomicrobium* sp. ($F_{2,6}$ = 22.43, P_{FDR} = 0.035) emerged from the GLMs as more abundant in U2 and U4 soils, respectively (Fig. 5).

Pedomicrobium sp. has been identified in soil samples as a heterotrophic bacterium that can oxidise manganese and iron (Rosenberg et al. 2014). Also, it's reported to be a metal-tolerant species and present in Cr-contaminated sites (Sheik et al. 2012; Araujo et al. 2023), in microcosm experiments it showed hydrocarbon-clastic capacities (de la Cueva et al. 2016), and was significantly predominant in soils contaminated with low-density polyester microplastics (LDPE-MPs) up to 7% w/w (Rong et al. 2021).

Gaiella sp. has been identified as a strict chemoorganotroph that can also be encountered in soil (Albuquerque and da Costa 2014). Its presence also seems to be positively correlated with the presence of polycyclic aromatic hydrocarbons (PAHs) in soils, but information on the ecological response of this bacterium to PAH contamination is still limited (Zhang et al. 2023).

The Venn diagram (Fig. 6a) revealed that site U3 harboured the highest proportion of unique ASVs (22%), suggesting a more distinct microbial community structure, potentially driven by site-specific environmental conditions or human impact, as a consequence of a higher influx of

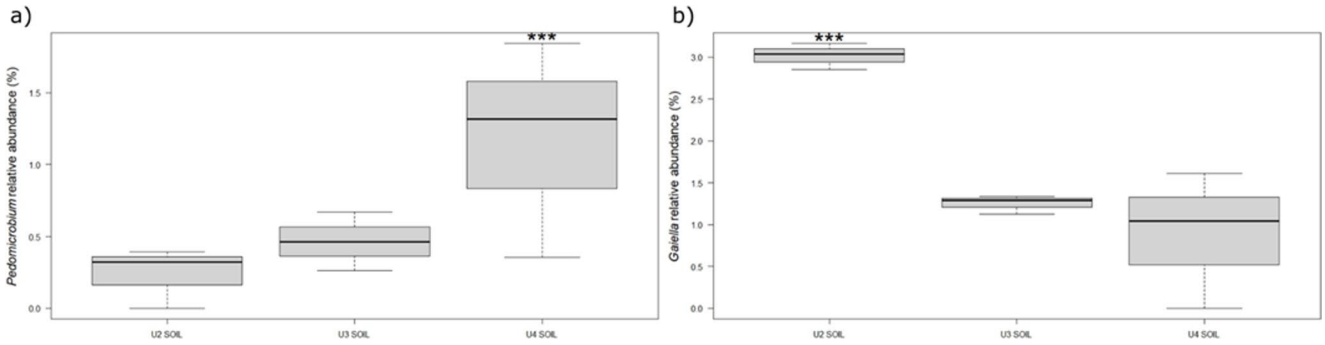

Fig. 5 Boxplot showing the statistical significance of the relative amplicon read abundances of (**a**) *Pedomicrobium sp.* and (**b**) *Gaiella sp.* in the different sampling areas ($n = 18$ replicates per green area). Asterisks denote significant differences to control (Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05)

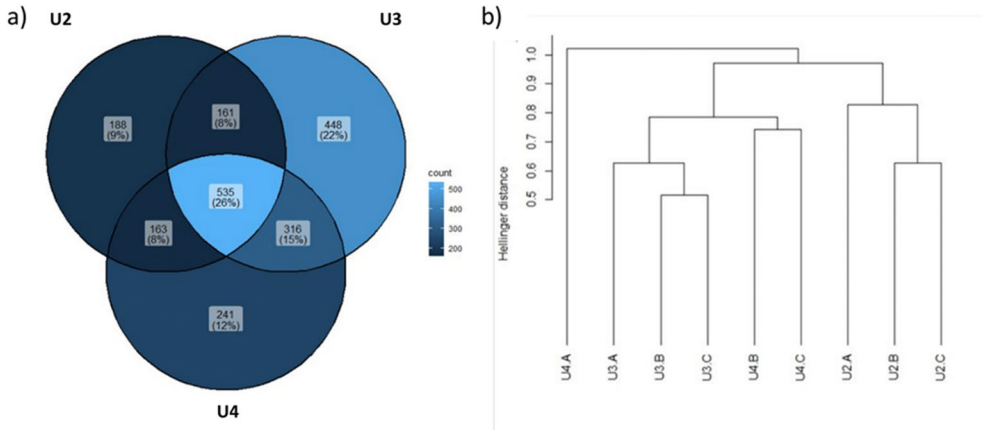

Fig. 6 (**a**) Venn diagram of similar shared ASVs for the three different areas ($n = 18$ replicates per green area). (**b**) Hierarchical cluster analysis on the Hellinger-transformed ASV table of the bacterial communities

Table 2 Pedofauna diversity refers to the structural (d-Margaled, D-Simpson, H-Shannon, AED, A/C ratio, Gini, Chao, number of ASV) and functional indices (QBS-ar) of soils from U2, U3, and U4 green areas. Values are expressed as mean ± standard error ($n = 18$ replicates per green are)

Sites	Group	d-Margalef	D-Simpson	H-Shannon	AED	A/C ratio	QBS-ar
U2	Pedofauna	3.3 (±0.10)	0.19 (±0.02)	2.1 (±0.10)	25.6 (±1.02)	1.40 (±0.52)	142 (±6.7)
U3	Pedofauna	2.2 (±0.23)	0.26 (±0.01)	1.7 (±0.05)	15.6 (±1.51)	0.49 (±0.01)	115 (±17.3)
U4	Pedofauna	2.1 (±0.42)	0.23 (±0.01)	1.8 (±0.06)	14.9 (±2.38)	0.48 (±0.04)	97 (±4.01)

students in these green areas. In contrast, U4 and U2 presented lower proportions of unique ASVs, at 12% and 9%, respectively. The overlap in community composition was minimal between U2 and the other sites (8% shared ASVs), whereas U3 and U4 exhibited a greater degree of similarity (15% shared ASVs). Importantly, 26% of ASVs were shared among all three sites, indicating a core microbiome potentially reflective of common ecological functions or environmental baselines across urban green areas.

Cluster analysis on the Hellinger-transformed ASV table of the bacterial communities (Fig. 6b) further supported this spatial differentiation, with samples from U3 and U4 predominantly clustering by site. Notably, U4A samples formed a distinct subgroup with a high heterogeneity when comparing all the replicates, separate from the remaining samples, while U2 samples diverged early from the other clusters (Fig. 6b). These patterns indicate a higher degree of similarity in both ASV richness and relative abundance between the U3 and U4 sites. This observation aligns with the Venn diagram results, which showed a higher proportion of shared ASVs between U3 and U4 (15%), reinforcing the potential use of shared microbial taxa as indicators of ecological similarity and site conditions.

Ecological indices

The utilisation of ecological indices (Table 2) facilitated the integration of diversity data, confirming a significant decline in edaphic invertebrates in U3 and U4 compared to U2 areas, whereas no significant differences were detected between U3 and U4 areas (Table SM8).

All structural and functional indices showed the highest level of invertebrate species, more widely distributed and belonging to edaphic groups with ecomorphological

Table 3 Bacterial diversity refers to the structural (H-Shannon, Gini, Chao, number of ASV) indices of soils from U2, U3, and U4 green areas. Values are expressed as mean ± standard error ($n = 18$ replicates per green area)

Sites	Group	H-Shannon	Gini	Chao	ASV
U2	Bacteria	5.75 (±0.26)	0.87 (±0.03)	778.59 (±268.44)	563 (±47.41)
U3	Bacteria	6.17 (±0.05)	0.8 (±0.01)	1487.3 (±204.65)	846.33 (±43.24)
U4	Bacteria	5.8 (±0.37)	0.85 (±0.05)	971.69 (±370.4)	623.33 (±192.3)

adaptations relevant to U2 soils. Conversely, the soils of U3 and U4 showed a worse condition in terms of diversity, with a dominance in generalist and tolerant edaphic species. These outcomes were corroborated and consistent with the results previously detected by behavioural tests. U4 soils, which had limited habitat function, showed the lowest diversity, and U3 soils also showed reduced diversity compared to U2 soils, despite displaying a higher quantified organic matter content. The results outlined from U3 also demonstrated that conducting avoidance or disaggregation tests alone would not be exhaustive for understanding alterations in soil diversity, as aggregation behaviour, a positive condition of ecological quality, occurred in U3 soils with low edaphic diversity, while avoidance tests itself for these soils would not have clarified whether it was movement inhibition or spontaneous attraction. It is therefore suggested that the combination of the two endpoints is essential for a correct environmental safety assessment of soil quality.

Conversely to the invertebrate diversity, analysis of alpha diversity indices revealed no statistical difference between the sampling areas in terms of bacterial communities (Table 3).

Discussion

The habitat function of urban soils was assessed through a combined approach between behavioural responses of soil fauna and biodiversity analyses, providing a comprehensive framework to evaluate ecosystem quality. This multilevel strategy enabled us to identify ecological indicators that reflect key aspects of soil community structure and potential habitat suitability, highlighting the added value of behavioural bioassays as early-warning tools. y. Our findings confirmed that behavioural responses of soil organisms, particularly in avoidance and disaggregation bioassays, serve as indicator tools for assessing soil quality, offering a rapid and cost-effective screening method. Unlike traditional ecotoxicological endpoints, behavioural responses integrate multiple environmental constraints and reflect the capacity of soils to support organismal activity, persistence, and ecological interactions. In this context, avoidance and disaggregation emerged as complementary indicators of habitat quality. Avoidance behaviour reflected the perception of unfavourable edaphic conditions and was expressed as active displacement away from stressed soils, resulting in reduced residence time and limited colonisation. Disaggregation responses, by contrast, revealed more subtle functional impairments through the fragmentation of social behaviour, particularly in gregarious taxa such as terrestrial isopods. Importantly, disaggregation did not necessarily imply complete abandonment of the soil, but rather a local redistribution of individuals, occurring both within stressed soils and in adjacent buffer zones, under suboptimal conditions. The choice of model organisms, including both soft-bodied (e.g., earthworms) and hard-bodied invertebrates (e.g., woodlice and springtails), was essential to capture a wide range of ecological sensitivities and behavioural responses. This selection allowed for a broader assessment of edaphic conditions, as these taxa differ in physiological tolerance, mobility, and habitat requirements (Menta and Remelli 2020). The inclusion of all three organism types within the broad soft- and hard-bodied categories was justified by the observed differences in responses, which were complementary rather than redundant. Notably, terrestrial isopods displayed the most distinct and sensitive behavioural patterns, likely due to their gregarious nature, which amplifies responses to environmental cues and enhances detectability of habitat quality differences. The utilisation of terrestrial isopods also facilitated the observation of variations in population structure through the disaggregation endpoints, which are not captured by conventional avoidance test (Federico et al. 2024). Consequently, these model organisms may represent a rapid assessment tool for soil quality to complement standard ecotoxicological assays.

Analysis of biodiversity provided quantitative confirmation of soil habitat functionality, as predicted by the behavioural tools. This consistency was corroborated by the study of invertebrate diversity, where the U2 soils were found to be those with no limited habitat functions and with the greatest diversity. The study of bacterial communities revealed no significant variations of the alpha diversity indexes. Although high bacterial alpha diversity is indicative of a healthy soil (Van Elsas et al. 2006; Hermans et al. 2017), it is expected that diversity indices do not show marked differences, as microbial communities may not follow the same basic rules of ecology as many edaphic organisms in response to a disturbance (Fierer et al. 2011; Lear et al. 2011). Microbial communities respond rapidly to environmental changes (Rutigliano et al. 2023), and show broad tolerance e for a range of stresses, such as temperature, pH, heavy

metal and radionuclide concentrations (Satyanarayana et al. 2005), allowing high levels of diversity even under extreme environmental conditions. These results suggest that behavioural bioassays may be more sensitive than microbial diversity metrics in detecting local-scale functional habitat degradation.

Conversely, invertebrate communities may be more directly affected by even small variations in edaphic and physico-chemical factors than bacterial communities, such as variations in water retention and pH, as well as the presence of chemical or physical contaminants (Menta and Remelli 2020). Specifically, sampled soils from U2 areas showed a high percentage of species sensitive to changes in soil conditions such as of Acarina, Protura, and Isopoda compared to the other green areas. Mites and Proturans are generally sensitive to mechanical stresses induced by trampling (Maraun et al. 2003), and deforestation (Toth et al. 2023). As a consequence of U2 isolation, the reduction of human disturbance may facilitate the increase in abundance of mites and proturans. Isopoda represent synanthropic species successfully adapted to urban soils (Vilisics et al. 2012; Hornung et al. 2018; Szlavecz et al. 2018), and their significant decrease in abundances in disturbed habitats, such as U3 and U4 soils, highlights their potential role as indicators of soil quality, as supported by previous studies (Paoletti and Hassall 1999; van Gestel et al. 2018). These outcomes suggest that the presence and abundance of Acarina, Protura and Isopoda could be indicative of the soil's ecological integrity, further emphasizing the importance of habitat preservation and management in urban green spaces.

As for the U2 soils, also for the U3 and U4 soils, the results of the behavioural tests were found to be consistent with those relating to invertebrate diversity, as well as with the percentage levels of WHC and SOM detected, the latter likely reflecting inputs from bars and restoration areas near the university refectory or the effects of soil compaction from foot traffic. In fact, U3 soils exhibited a higher abundance of species sensitive to soil moisture loss, such as collembolans (Hopkin 1997), or symphylans (Edwards 1961), and species sensitive to organic matter content, such as diplopoda, and earthworms (Lapied et al. 2009; Huerta et al. 2013). These results indicate that the abundance of springtails, symphylans, and earthworms may be indicators of soils with higher moisture and organic matter content. The high percentage of SOM likely induced attractive behaviour in all populations of model organisms tested in behavioural bioassays, as well as increasing the gregarious behaviour of terrestrial isopods. It is interesting to note that the SOM increases in U3 was higher despite a decline in faunal diversity. This may be an example of the "enrichment paradox" (Rosenzweig 1971), where an increase in productivity does not correspond to an increase in fauna diversity. However, the effect of additional organic matter on soil biodiversity depends on its quality and structure, and the present study does not allow for verification of mechanistic explanations of this effect, which would require more detailed studies on interactions between populations.

Conversely, U4 soils exhibited the most restricted levels of both structural and functional diversity. It is noteworthy that behavioural assessments proved to be more discerning indicators of habitat functional limitations in comparison to bacterial analyses. These soils showed a high abundance of Hymenoptera, specifically of ants (Formicidae). The higher abundance of ants can be attributed to their competitive behaviour for food and territory (Trainello, 1989; Duma 2003). Cakir (2019) showed that ants reduce and replace Collembola and Protura in arid and semi-arid environments. Furthermore, ants modify soil texture, increasing porosity and reducing water retention capacity (Cammeraat et al. 2002; Frouz and Jilková 2008), which affects edaphic species requiring higher moisture, such as Collembola. Furthermore, the family Formicidae consists of vagile species, and this could be a possible explanation for their low sensitivity to current soil disturbances compared to other taxa (Remelli et al. 2024). The results suggest that an increasing abundance of ants may be an indicator of low diversity. At the same time, it cannot be excluded that their abundance may depend on an induced recruitment effect induced by an alteration of the ecological conditions of the U4 soils, such as the presence of contaminants or fertilisers.

In light of these findings, it is necessary to recognize the spatial and temporal limitations of our biodiversity assessments. Sample size and representativeness are essential parameters for reliable analysis and that the implementation of seasonally repeated sampling is crucial to improve the resolution of biodiversity dynamics and trends, especially in an explicit spatial context (Hillebrand et al. 2018). Specifically, arthropod communities are affected by seasonal variations in both highly and slightly polluted soils, exhibiting distinct responses to the same site when sampled at different times (Santorufo et al. 2014). Despite these constraints, the aim of this study was to undertake a first attempt at proposing a framework that links behavioural early-warning responses to biodiversity loss. This endeavour was undertaken in order to address the priorities of the European Commission (EC 2025b), which highlights the current misalignment between biodiversity loss metrics and laboratory-based ecotoxicological assessments. We acknowledge and support the need for future studies incorporating a larger number of samples and seasonal sampling, which will be essential to strengthen the validity and robustness of these preliminary results.

Conclusion

This work represents a first attempt to use behavioural responses as a tool to support soil biodiversity assessment within a multilevel framework. By integrating dispersal traits of different hard- and soft-bodied edaphic organisms, along with fragmentation traits of gregarious species such as terrestrial isopods, it is possible to identify soils potentially subject to invertebrate diversity loss. This approach allows for the prioritization of economic and management interventions in a targeted and efficient manner for assessing urban ecosystems. Future studies will need to validate this framework across different environmental contexts (i.e. agriculture, forest, wetland, and prairie soils), at larger spatial scales and over multiple sampling periods, to consolidate its applicability and robustness for urban soil health assessment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11252-026-01908-6.

Acknowledgements We acknowledge Dr Valerio Orioli for technical assistance with the extraction of the edaphic invertebrate, Dr Davide Calvi for his contribution to the sampling activities, and Dr. Samuele Saccardi for his contribution to the identification of the edaphic invertebrate.

Author contributions Lorenzo Federico (Conceptualization, Visualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review and editing), Valeria Tatangelo (Data curation, Investigation), Francesca Pittino (Data curation, Formal analysis), Claudia Russo (Investigation), Emanuele Vegini (Investigation), Sandra Citterio (Supervision, Resources, Funding acquisition, Writing – review and editing), Andrea Franzetti (Supervision, Resources, Writing – review and editing), Sara Villa (Conceptualization, Resources, Validation, Data curation, Supervision, Project administration, Writing – original draft, Writing – reviewer & editing).

Funding Open access funding provided by Università degli Studi di Milano - Bicocca within the CRUI-CARE Agreement. This work was supported by the MUSA—Multilayered Urban Sustainability Action—project (contract number ECS 000037) and funded by the European Union—NextGenerationEU, under the National Recovery and Resilience Plan (NRRP) Mission 4 Component 2 Investment Line 1.5: Strengthening of research structures and the creation of R&D "innovation ecosystems", set up by "territorial leaders in R&D".

Data availability The data that support the findings of this study are openly available in ZENODO at https://zenodo.org/records/17367638.

Declarations

Supplementary Information Below is the link to the electronic supplementary material.

Competing interests The authors declare no competing interests.

References

Albuquerque L, da Costa MS (2014) The family Gaiellaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30138-4_394

Araujo ASF, Miranda ARL, de Araujo Pereira AP, de Melo WJ, Melo VMM, Ventura SH, Brito Junior ES, de Valente Meiros E, Araujo FF, Mendes LW (2023) Microbial communities in the rhizosphere of maize and cowpea respond differently to chromium contamination. Chemosphere 313:137417. https://doi.org/10.1016/j.chemosphere.2022.137417

Bachelier G (1986) La vie animale dans le sol. O.R.S.T.O.M., Paris, 171–196

Bowler DE, Buyung-Ali L, Knight TM, Pullin AS (2010) Urban greening to cool towns and cities: a systematic review of the empirical evidence. Landsc Urban Plan 97(3):147–155. https://doi.org/10.1016/j.landurbplan.2010.05.006

Çakır M (2019) The negative effect of wood ants (*Formica rufa*) on microarthropod density and soil biological quality in a semi-arid pine forest. Pedobiologia 77:150593. https://doi.org/10.1016/j.pedobi.2019.150593

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Callow D, May P, Johnstone DM (2018) Tree vitality assessment in urban landscapes. Forests 9(5):279. https://doi.org/10.3390/f9050279

Cammeraat LH, Willott SJ, Compton SG, Incoll LD (2002) The effects of ants' nests on the physical, chemical and hydrological properties of a rangeland soil in semi-arid Spain. Geoderma 105(1–2):1–20. https://doi.org/10.1016/S0016-7061(01)00085-4

Chao A (1984) Non-parametric estimation of the number of classes in a population. Scand J Stat 11:265–270. https://doi.org/10.2307/4615964

Coyle DR, Nagendra UJ, Taylor MK, Campbell JH, Cunard CE, Joslin AH, Mundepid A, Phillips CA, Callaham MA Jr (2017) Soil fauna responses to natural disturbances, invasive species, and global climate change: current state of the science and a call to action. Soil Biol Biochem 110:116–133. https://doi.org/10.1016/j.soilbio.2017.03.008

de la Cueva SC, Rodríguez CH, Cruz NOS, Contreras JAR, Miranda JL (2016) Changes in bacterial populations during bioremediation of soil contaminated with petroleum hydrocarbons. WASP 227:1–12. https://doi.org/10.1007/s11270-016-2789-z

Duma I (2003) The impact of red wood ants *Formica Rufa* on the distribution of invertebrate fauna from the forest's floor. AWUT-SerBio 1011:121–130. https://www.jstor.org/stable/4223297

EC (2025b) European Commission: Joint Research Centre, Baccaro, M., Focks, A., Worth, A., Chinchio, E., Dubois, G., Robuchon, M., Carletti, A., Bernasconi, C. and Bopp, S., Protection of biodiversity as the ultimate goal of environmental safety assessment: how does chemical pollution affect biodiversity, Publications Office of the European Union, Luxembourg, 2025. https://doi.org/10.2760/1215173

EC (2021) European Commission, 2021. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions Pathway to a Healthy Planet for All EU Action Plan: Towards Zero Pollution for Air, Water and Soil. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:52021DC0400

EC, Commission E (2025a) 2025a. P9_TA(2024)0204 – Soil Monitoring and Resilience (Soil Monitoring Directive) – European Parliament legislative resolution of 10 April 2024 on the proposal for a directive of the European Parliament and of the Council on Soil Monitoring and Resilience (Soil Monitoring Law) (COM(2023)0416 – C9-0234/2023–2023/0232(COD)) (Ordinary legislative procedure: first reading) http://data.europa.eu/eli/C/2025/1312/oj

Edwards CA (1961) The ecology of symphyla part III. Factors controlling soil distributions. Entomol Exp Appl 4(4):239–256. https://doi.org/10.1111/j.1570-7458.1961.tb02139.x

EU, European, Parliament, Council of the European Union, 2024. Regulation (EU) 2024/1991 of the European Parliament and of the Council of 24 June 2024 on nature restoration and amending Regulation (EU) 2022/869 (Text with EEA relevance). Off J Eur, Union (2024) Brussels (BE). http://data.europa.eu/eli/reg/2024/1991/oj

Federico L, Monti GS, Loureiro S, Villa S (2024) Disaggregation behavior in the terrestrial isopod *Porcellionides pruinosus* as a new ecotoxicological endpoint for assessing infochemical disrupting activity. Ecol Indic 167:112602. https://doi.org/10.1016/j.ecolind.2024.112602

Fierer N, McCain CM, Meir P, Zimmerman M, Rapp JM, Silman MR, Knight R (2011) Microbes do not follow the elevational diversity patterns of plants and animals. Ecology 92:797–804. https://doi.org/10.1890/10-1170.1

Frouz J, Jilková V (2008) The effect of ants on soil properties and processes (Hymenoptera: Formicidae). Myrmecol News 11(11):191–199

Gainer A, Owojori OJ, Maboeta M (2022) Use of soil invertebrate avoidance tests as an emerging tool in soil ecotoxicology. Rev Environ Contam Toxicol 260(1):2. https://doi.org/10.1007/s44169-021-00004-4

Gandolfi I, Canedoli C, Rosatelli A, Covino S, Cappelletti D, Sebastiani B, Tatangelo V, Corengia T, Pittino F, Padoa-Schioppa E, Báez-Matus X, Hernández L, Seeger M, Saati-Santamaría Z, García-Fraile P, López-Mondéjar R, Ambrosini R, Papacchini M, Franzetti A (2024) Microbiomes of urban trees: unveiling contributions to atmospheric pollution mitigation. Front Microbiol 15:1470376. https://doi.org/10.3389/fmicb.2024.1470376

Gatti RC, Amoroso N, Monaco A (2020) Estimating and comparing biodiversity with a single universal metric. Ecol Modell 424:109020. https://doi.org/10.1016/j.ecolmodel.2020.109020

Gini CW (1912) Variability and mutability, contribution to the study of statistical distributions and relations. Studi Economico-Giuridici della R. Università de Cagliari

Gottardini E, Cristofolini F, Cristofori A, Pollastrini M, Ferretti M (2016) Misura della fluorescenza della clorofilla A, contenuto di clorofilla e tratti fogliari: campionamento, raccolta e misurazioni: guida per studi in campo. IT. http://hdl.handle.net/10449/38027

Hermans SM, Buckley HL, Case BS, Curran-Cournane F, Taylor M, Lear G (2017) Bacteria as emerging indicators of soil condition. Appl Environ Microbiol 83(1):e02826-16. https://doi.org/10.1128/AEM.02826-16

Hillebrand H, Blasius B, Borer ET, Chase JM, Downing JA, Eriksson BK, Filstrup CT, Harpole WS, Hodapp D, Larsen S, Lewandowska AM, Seabloom EW, de Van Waal DB, Ryabov AB (2018) Biodiversity change is uncoupled from species richness trends: consequences for conservation and monitoring. J Appl Ecol 55(1):169–184. https://doi.org/10.1111/1365-2664.12959

Hopkin SP (1997) Biology of the springtails:(Insecta: Collembola). OUP Oxford

Hornung E, Kásler A, Tóth Z (2018) The role of urban forest patches in maintaining isopod diversity (Oniscidea). ZooKeys 801:371. https://doi.org/10.3897/zookeys.801.22829

Huerta E, Brunet D, Velazquez E, Lavelle P (2013) Identifying earthworm's organic matter signatures by near infrared spectroscopy in different land-use systems in Tabasco, Mexico. Appl Soil Ecol 69:49–55. https://doi.org/10.1016/j.apsoil.2013.01.015

ISO (2020b) Soil quality - Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Part 2: Test with collembolans (*Folsomia candida*). ISO 7512-2:2020

ISO (2020a) Soil quality - Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*). ISO 7512-2:2020

Lapied E, Nahmani J, Rousseau GX (2009) Influence of texture and amendments on soil properties and earthworm communities. Appl Soil Ecol 43(2–3):241–249. https://doi.org/10.1016/j.apsoil.2009.08.004

Lear G, Dopheide A, Ancion P, Lewis GD (2011) A comparison of bacterial, ciliate and macroinvertebrate indicators of stream ecological health. Aquat Ecol 45(4):517–527. https://doi.org/10.1007/s10452-011-9372-x

Linden DR, Hendrix PF, Coleman DC, van Vliet PC (1994) Faunal indicators of soil quality. Defining soil quality for a sustainable environment, vol 35. pp 91–106. https://doi.org/10.2136/sssaspecpub35.c6

Maraun M, Salamon JA, Schneider K, Schaefer M, Scheu S (2003) Oribatid mite and collembolan diversity, density and community structure in a moder beech forest (*Fagus sylvatica*): effects of mechanical perturbations. Soil Biol Biochem 35(10):1387–1394. https://doi.org/10.1016/S0038-0717(03)00218-9

Margalef R (1958) Temporal succession and spatial heterogeneity in phytoplankton. Perspectives in Marine Biology, edited by A. A. Buzzati-Traverso, Berkeley: University of California Press. https://doi.org/10.1525/9780520350281-024

Maria VL, Santos J, Prodana M, Cardoso DN, Morgado RG, Amorim MJ, Barreto A (2024) Toxicity mechanisms of plastic nanoparticles in three terrestrial species: antioxidant system imbalance and neurotoxicity. Nanotoxicology 18(3):299–313. https://doi.org/10.1080/17435390.2024.2358781

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51(345):659–668. https://doi.org/10.1093/jexbot/51.345.659

McSorley R (2000) Sampling and extraction techniques for nematodes. Techniques in nematode ecology. eds T Wheeler, T Forge, E Caswell-Chen. Hyattsville, MD: Society of Nematologists

Menta C, Remelli S (2020) Soil health and arthropods: from complex system to worthwhile investigation. Insects 11:54. https://doi.org/10.3390/insects1101005

Menta C, Conti FD, Pinto S, Bodini A (2018) Soil biological quality index (QBS-ar): 15 years of application at global scale. Ecol Indic 85:773–780. https://doi.org/10.1016/j.ecolind.2017.11.030

Oliveira M, Cardoso DN, Soares AMVM, Loureiro S (2015) Effects of short-term exposure to fluoxetine and carbamazepine to the collembolan *Folsomia candida*. Chemosphere 120:86–91. https://doi.org/10.1016/j.chemosphere.2014.06.038

OriginLab Pro 2021b software. url: www.OriginLab.com/2021b

Paoletti MG, Hassall M (1999) Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agric Ecosyst Environ 74(1–3):157–165. https://doi.org/10.1016/S0167-8809(99)00035-3

Parisi V, Menta C, Gardi C, Jacomini C, Mozzanica E (2005) Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. Agric Ecosyst Environ 105(1–2):323–333. https://doi.org/10.1016/j.agee.2004.02.002

Pereira CS, Lopes I, Abrantes I, Sousa JP, Chelinho S (2019) Salinization effects on coastal ecosystems: a terrestrial model ecosystem approach. Philos Trans R Soc Lond B Biol Sci 374(1764):20180251. https://doi.org/10.1098/rstb.2018.0251

Picot X (2004) Thermal comfort in urban spaces: impact of vegetation growth: case study: Piazza Della Scienza, Milan, Italy. Energy Build 36(4):329–334. https://doi.org/10.1016/j.enbuild.2004.01.044

R Core Team (2022) R foundation for statistical computing. Chin J Hepatol 30:9–20

Remelli S, Danise T, Galli L, Menta C (2024) Soil arthropods in bioindication and ecotoxicological approach: the case of the extreme environment Mefite (Ansanto Valley, Southern Italy). Heliyon. https://doi.org/10.1016/j.heliyon.2024.e36342

Rong L, Zhao L, Zhao L, Cheng Z, Yao Y, Yuan C, Wang L, Sun H (2021) LDPE microplastics affect soil microbial communities and nitrogen cycling. Sci Total Environ 773:145640. https://doi.org/10.1016/j.scitotenv.2021.145640

Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (2014) The Prokaryotes – Alphaproteobacteria and Betaproteobacteria. Springer Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30138-4_394

Rosenzweig ML (1971) Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. Science 171(3969):385–387. https://doi.org/10.1126/science.171.3969.385

Rutigliano FA, Marzaioli R, Grilli E, Coppola E, Castaldi S (2023) Microbial, physical and chemical indicators together reveal soil health changes related to land cover types in the Southern European sites under desertification risk. Pedobiologia 99:150894. https://doi.org/10.1016/j.pedobi.2023.150894

Sachs JD (2012) From millennium development goals to sustainable development goals. Lancet 379(9832):2206–2211. https://doi.org/10.1016/S0140-6736(12)60685-0

Santorufo L, Van Gestel CA, Rocco A, Maisto G (2012) Soil invertebrates as bioindicators of urban soil quality. Environ Poll 161:57–63. https://doi.org/10.1016/j.envpol.2011.09.042

Santorufo L, Van Gestel CA, Maisto G (2014) Sampling season affects conclusions on soil arthropod community structure responses to metal pollution in mediterranean urban soils. Geoderma 226:47–53. https://doi.org/10.1016/j.geoderma.2014.02.001

Satyanarayana T, Raghukumar C, Shivaji S (2005) Extremophilic microbes: diversity and perspectives. Curr Sci 89:78–90. https://www.jstor.org/stable/24110434

Schloter M, Dilly O, Munch JC (2003) Indicators for evaluating soil quality. Agric Ecosyst Environ 98(1–3):255–262. https://doi.org/10.1016/S0167-8809(03)00085-9

Sepúlveda P, Johnstone M, D (2018) A novel way of assessing plant vitality in urban trees. Forests 10(1):2. https://doi.org/10.3390/f10010002

Shannon CE (1948) A mathematical theory of communication. BSTJ 27(3):379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Sheik CS, Mitchell TW, Rizvi FZ, Rehman Y, Faisal M, Hasnain S, McInerney MJ, Krumholz LR (2012) Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. PLoS ONE 7(6):e40059. https://doi.org/10.1371/journal.pone.0040059

Simpson EH (1949) Measurement of diversity. Nature 163(4148):688–688. https://doi.org/10.1038/163688a0

Szlavecz K, Vilisics F, Tóth Z, Hornung E (2018) Terrestrial isopods in urban environments: an overview. ZooKeys 801:97. https://doi.org/10.3897/zookeys.801.29580

Tóth Z, Dombos M, Hornung E (2023) Urban soil quality deteriorates even with low heavy metal levels: an arthropod-based multi-indices approach. Ecol Appl 33(4):e2848. https://doi.org/10.1002/eap.2848

Traniello JF (1989) Foraging strategies of ants. Annu Rev Entomol 34(1):191–210. https://doi.org/10.1146/annurev.en.34.010189.001203

UNEP and FAO (2020) Strategy of the UN decade on ecosystem Restoration. Nairobi and Rome. Draft 6 February 2020

Van Elsas JD, Trevors JT, Jansson JK, Nannipieri P (2006) Modern soil microbiology. CRC. https://doi.org/10.1201/9781420015201

van Gestel CA, Loureiro S, Zidar P (2018) Terrestrial isopods as model organisms in soil ecotoxicology: a review. ZooKeys 801:127. https://doi.org/10.3897/zookeys.801.21970

Vieira DCS, Yunta F, Baragaño D, Evrard O, Reiff T, Silva V, De La Torre A, Zhang C, Panagos P, Jones A, Wojda P (2024) Soil pollution in the European Union–an outlook. Environ Sci Policy 161:103876. https://doi.org/10.1016/j.envsci.2024.103876

Vilisics F, Bogyó D, Sattler T, Moretti M (2012) Occurrence and assemblage composition of millipedes (Myriapoda, Diplopoda) and terrestrial isopods (Crustacea, Isopoda, Oniscidea) in urban areas of Switzerland. ZooKeys 176:199. https://doi.org/10.3897/zookeys.176.2153

Zhang Q, Gao M, Sun X, Wang Y, Yuan C, Sun H (2023) Nationwide distribution of polycyclic aromatic hydrocarbons in soil of China and the association with bacterial community. J Environ Sci 128:1–11. https://doi.org/10.1016/j.jes.2022.07.026