

Landscape and Urban Planning

Compositional novelty of plant, fungal and bacterial communities across urban habitats

--Manuscript Draft--

Manuscript Number:	LANDUP-D-25-00741R1
Article Type:	Research Paper
Section/Category:	Habitat, Wildlife and Biodiversity
Keywords:	Urban biodiversity, soil fungi, soil bacteria, DNA metabarcoding, soil pollution, novel ecosystems
Corresponding Author:	Eduardo Fernández-Pascual University of Oviedo SPAIN
First Author:	Eduardo Fernández-Pascual
Order of Authors:	Eduardo Fernández-Pascual Zuzana Ferencova Víctor González-García Borja Jiménez-Alfaro
Abstract:	<p>Understanding urban community novelty can help to predict the rewilding potential of vacant urban spaces, facilitating their integration into biodiverse cities. We tested the hypothesis that cities are composed of different degrees of ecological novelty by studying four urban habitats with differing degrees of management and human legacy: park lawns, roadsides, residential vacant lots, and industrial vacant lots. We focused on community compositional novelty, by comparing the plant, fungal and bacterial species composition between urban habitats and two reference pre-urban habitats: forests and meadows. We used a compositional novelty index based on multidimensional ordination, which is straightforward to calculate and only requires species co-occurrence data for urban and reference habitats. As expected, (1) plants displayed the highest novelty and bacteria the lowest; (2) urban communities were markedly different from forests and relatively more similar to meadows; and (3) compositional novelty was highest in industrial lots. Managed park lawns, which we had expected to be highly novel, were relatively close to hay meadows. The lowest novelty was recorded in residential vacant lots, which had biological communities that more closely resembled those of pre-urban habitats. Our results highlight the effect of habitat type as a major driver of urban community composition and novelty. This suggests that city biodiversity can be enhanced by an integrative approach to the urban landscape that favors habitat heterogeneity by passive rewilding of park lawns, non-intervention on residential vacant lots, direct restoration of industrial lots, and conservation of natural and agricultural habitat remnants as sources of native species.</p>
Response to Reviewers:	

Dear editors,

We submit the manuscript “Compositional novelty of plant, fungal and bacterial communities across urban habitats” to be considered for publication in Landscape and Urban Planning.

We test the hypothesis that cities are composed of different degrees of ecological novelty by studying four urban habitats with differing degrees of management and human legacy; and comparing them with two reference pre-urban habitats.

We use a compositional novelty index based on multidimensional ordination, which is straightforward to calculate and only requires species co-occurrence data for urban and reference habitats.

Our results support an integrative approach to urban landscape management that favors habitat heterogeneity by passive rewilding of managed park lawns, non-intervention on residential vacant lots, direct restoration of industrial vacant lots, and conservation of natural and agricultural habitat remnants as sources of native species.

Kind regards,

The authors

Compositional novelty of plant, fungal and bacterial communities across urban habitats

Editor

I note that Reviewer 1 and Reviewer 2 have conflicting assessments on your analyses. I agree with Reviewer 1 in that there are issues to address in your revision, and R1 provides detailed ways to move forward. In addition to addressing Reviewer comments, please also include 1-2 sentences in your Abstract that provides the context of the study. These first sentences usually summarize the Introduction. Starting the abstract with the hypotheses does not provide the background of the study.

Thank you for reviewing our manuscript and for providing directions to improve it. We have prepared a new version based on the suggestions of both reviewers. Among other things, we have conducted additional tests to assess the issues raised by reviewer 1. We note that, overall, the additional tests did not change the output and the interpretation of the results. We have also revised the abstract following your advice. During resubmission, we have uploaded a track-changes version of the manuscript as a “supporting file”.

Reviewer 1

This paper offers an intriguing study on assessing community novelty for multiple taxa in urban habitats. The authors use ordination methods to evaluate the differences between communities in urban habitats and those in natural and semi-natural settings, aiming to assess the novelty of urban communities. Their findings indicate that novelty is highest for plant communities and lowest for soil bacteria, with industrial vacant lots exhibiting the highest novelty among urban habitats.

Thank you for your positive evaluation and your suggestions for improving the manuscript.

The NMDS (Non-Metric Multidimensional Scaling) method should be carefully considered for ordination analysis in your case. Since NMDS employs an iterative algorithm to seek optimal solutions, the results may vary across different computational runs, leading to inconsistencies in ordination outcomes. Therefore, it is advisable to consider alternative ordination methods based on eigenvalue decomposition.

The referee is right in his description of NMDS. Following his advice, we tested the alternative of using a method based on eigenvalue decomposition: Principal Component Analysis (PCA) with Hellinger transformation. Using one method or the other did not change the outcome and interpretation of the analysis (neither the pattern of novelty across taxa,

urban habitats and reference habitats; nor the statistical significance of the LMM model). Therefore, we decided to use NMDS for the final presentation in the manuscript, since NMDS makes less assumptions about data and can be applied to a wider variety of datasets. We explain this in the methods section (lines 197-216). We also note that, although in the manuscript and figures we are reporting the output of a specific set of NMDS runs, during analysis we repeated the runs many times and the results never changed significantly; this supports that the results are robust despite the iterative nature of NMDS.

Additionally, the community data used for different biological groups are incompatible: plant data are based on abundance, while fungal and bacterial data use presence/absence metrics, resulting in a lack of comparability across taxa.

The referee is right, and thus we have changed the analysis by transforming plant cover into presence/absence, so the three taxa can be compared. We note that the output and interpretation of results have not been altered by this change.

Furthermore, critical details of the NMDS analysis—such as the number of axes used, the specific distance metric applied (e.g., Bray-Curtis), and the stress value (a key indicator of model fit)—were not adequately documented in the text.

We agree, and we have expanded the methods to provide a full report of NMDS including distance metric, number of axes and stress values (lines 208-216).

The methodology mentions the use of a Generalized Mixed Model, but the corresponding results are not reported. If the authors indeed used this method, they need to provide a more detailed description of the analysis process, such as the total sample size, the distribution family used, and whether model selection was conducted.

We thank the reviewer for spotting a mistake in our text. We did not use a GLM, but a Linear Mixed Model (i.e. Gaussian family). We did this after checking that LMM assumptions were met, as described in the methods section. We did not conduct model selection. We report model results (F and p values) in the text of the results section (lines 335-350). We have included a sentence about sample size in the methods (lines 221-223).

The paper employs PCA to reduce the dimensionality of soil physicochemical variables, but subsequently selects only three factors with the highest loadings on the first three axes for further analysis. Why not use the first three axes directly for subsequent analysis? Additionally, the explanatory rates of the first three PCA axes are not described.

Following the reviewer's advice, we now use the three axes for subsequent analysis. The only change this has caused in the results is that the first axis (corresponding to soil heavy metals) now has a significant fit with the NMDS ordination of the plant communities. We are not certain of what is meant by "explanatory rates of the first three PCA axes". We describe the contribution of each axis to the variability in the results section (lines 235, 241, 247).

The limited sample size of 30 plots undermines the robustness and reliability of the study's findings.

We believe that model significance indicates that the sample size was robust enough to provide statistical power to test the hypotheses of this study.

Furthermore, while natural forests were surveyed in the surrounding areas, the urban habitat sampling primarily concentrated on grassland vegetation, neglecting forest vegetation within urban contexts.

In the study city, there is no urban forest vegetation, except for natural forest remnants in the city's fringes. The level of management does not allow for succession of urban lots to proceed to any stage dominated by trees.

The Discussion section lacks depth, merely reiterating results without offering substantive interpretation. Additionally, the section titled "Widespread Soil Contamination Across Urban Habitats" strays from the central focus of the study.

We have revised the discussion and removed the section on soil contamination, following the reviewer's suggestion. In the discussion, there is a brief recap of the results for the benefit of the reader, but this is followed by their interpretation in light of the original hypotheses and the literature. Specifically, we believe that the discussion provides substantive interpretation regarding how our results should translate into management actions for each habitat type (see for example lines 414-419).

Several minor comments are detailed in the annotated manuscript.

We have addressed the minor comments included in the annotated PDF.

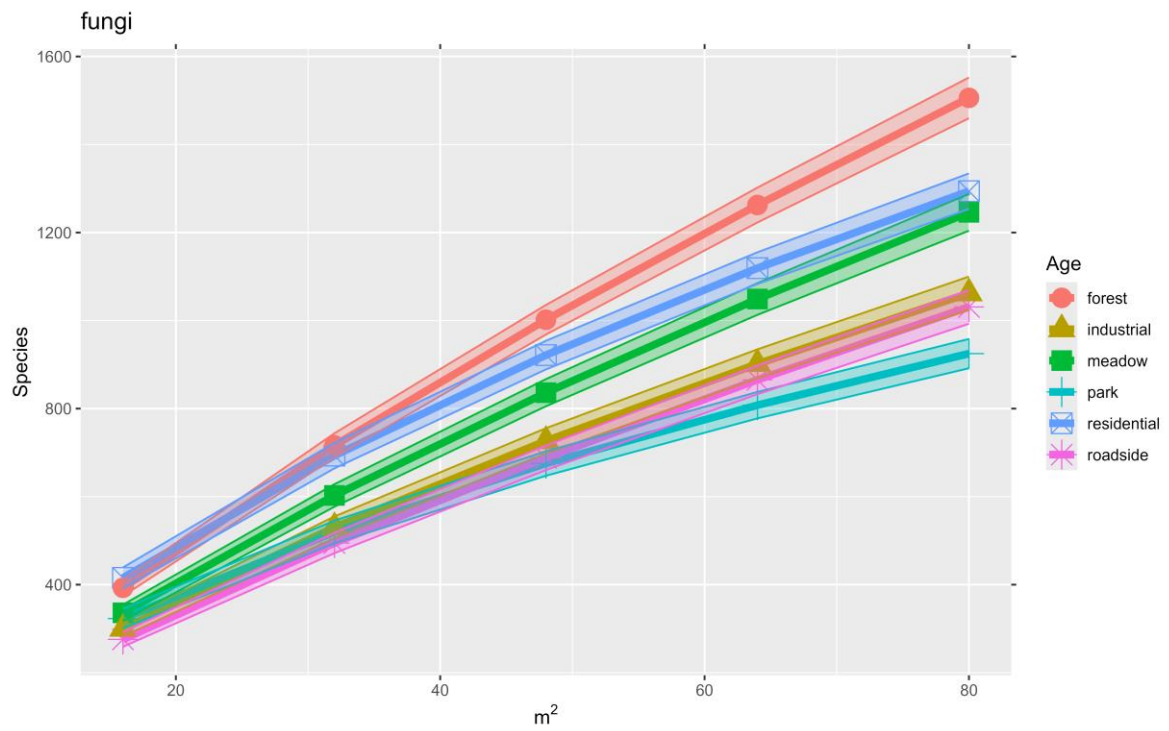
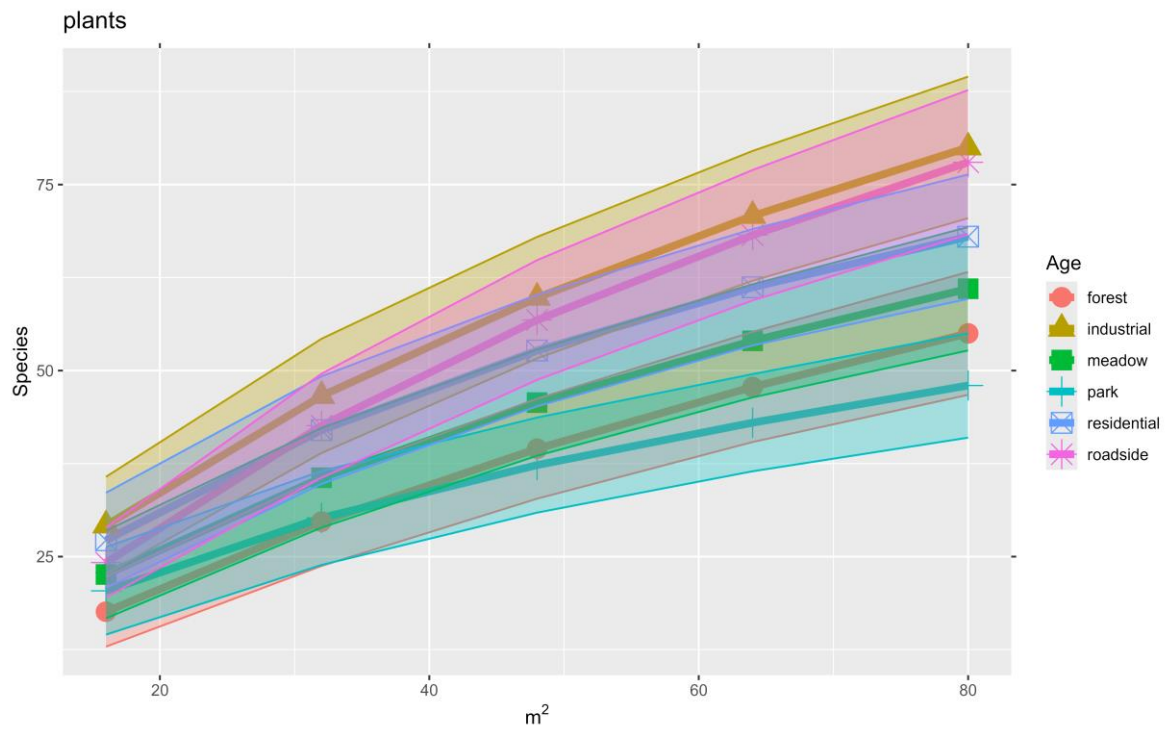
Reviewer 2

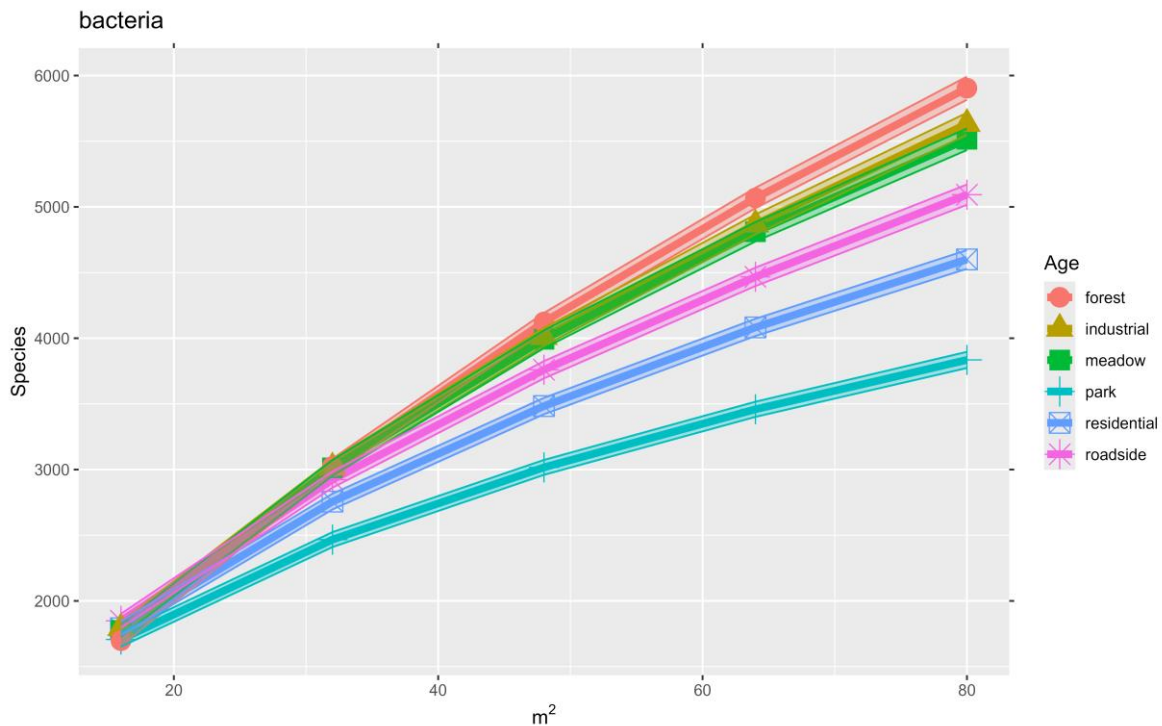
The paper describes the outcome of research conducted to characterize and better understand the diversity and compositional novelty of urban habitats with respect to their plant, fungal and bacterial communities. Although many studies have explored plant diversity associated with different habitats, this study is original in its investigation of the composition, diversity and relative novelty of not only plant communities, but also the soil microbiome in a range of urban habitats. The composition of these urban habitats is compared with remnant forest and meadow sites in the study region. Although there are relatively few investigations of bacterial and fungal community composition, the authors do cite other relevant work in the field. The authors also noted the motivation and significance of the work to urban restoration efforts in the introduction and returned to discuss their results in the light of restoration and rewilding in the discussion section. Most of the methods used in data acquisition and statistical analyses are appropriate to the investigation and results are reported clearly in the text and in the figures. The one exception is the lack of description of methods used to determine “Naturalness”, as noted below. The manuscript is quite well written. I found very few errors and had only a few questions after reading the article, as noted below.

Thank you for your positive consideration of our work.

Do you think that you were able to sample a sufficiently large number of replicates for each habitat type that you are approaching an understanding of the actual alpha diversity of these habitats (i.e., where are you on the species-area curve with respect to sampling the urban habitats you have chosen to study? What might you be able to discern from your data about the potential number of species that might be found if additional sampling had been conducted?

We have checked species-area curves (see below) and our sampling is not sufficient to cover the whole species diversity of the studied habitats. There are indeed additional data to support this: an iNaturalist project on the flora of the city currently has > 2,500 research-grade observations of c. 460 vascular plant species; our survey detected 216 species. Nonetheless, we note that it was not our goal to survey the entire biodiversity of the habitats, but to compare them. With this goal in mind, we have the same number of plots per habitat, of the same size.





The methods used to determine the level of “Naturalness” of each habitat are not described. In Figure 1, I am confused by the assignment of Industrial habitats to “high naturalness” as well as “high novelty”. While these habitats were species rich, they were rich in exotic and invasive species as well as native species. This seems to me to be a state of lower naturalness, despite the high diversity. Please clarify the methods used to estimate the “degree of ecosystem self-organization”, at the very least, cite other works whose methods you are using to determine “naturalness”.

We are following the terminology by Kowarik (see for example <https://doi.org/10.1016/j.ufug.2017.05.017>). According to this terminology, a community can be rich in introduced species (“novel”) but also “natural” in the sense that the species in the assemblage have arrived with little human intervention. Of course, the species have arrived to the regional species pool by human means, but their occurrence in the local community is spontaneous. For example, the community in a park lawn is less “natural” than the community in an abandoned industrial site, because the community in the park lawn is periodically affected by seeding, watering, fertilization, etc.; while the community in the industrial site is left to follow ecological succession for several years (until the site is

“cleared” out). We describe this and provide the reference in the introduction, paragraph 3 (lines 61-71). We note however that this is a conceptualization which we use for an a priori characterization of the habitats, rather than a methodology.

Page 18 line 6 – Use “As” rather than “Like” to start the sentence.

Changed as indicated.

I was taught that sentences should not begin with an acronym, such as sentences beginning with “NMDS...” or “DNA ...” . However, I realize that in recent times, fewer journals seem to be adhering to this grammatical rule. I will defer to the editor to determine whether the authors should revise their sentences to avoid having to begin sentences with an acronym.

We have revised the text to avoid starting sentences with acronyms.

Figure 4 A. The color for the Residential box in the box plot seems to be incorrect, at least it does not match the color scheme in the other figures- shouldn't it be teal blue and not purple?

The same color scheme is followed in all figures, however, in the case of Figure 4A, the residential box is so narrow that the black line which marks the median value hides the blue color of the box, and gives the impression of being purple when rendered at the final resolution.

Line 5-6 on page 14- To improve clarity, revise this sentence to : “As previously indicated by PCA, organic matter was the only soil property with a clear pattern of variation by habitat: a decrease from pre-urban to urban habitats (Figure 2B).

Changed as suggested.

- A compositional novelty index based on multidimensional ordination is straightforward to calculate and only requires species co-occurrence data for urban and reference pre-urban habitats.
- Novelty was the highest for plant communities and the lowest for soil bacteria.
- Urban communities were markedly different from forest communities and relatively more like meadow communities.
- Compositional novelty was highest in industrial vacant lots, and lowest in residential vacant lots.
- Park lawns were relatively close in composition to hay meadows.

Compositional novelty of plant, fungal and bacterial communities across urban habitats

Running title: Compositional novelty across urban habitats

Eduardo Fernández-Pascual^{1,2,a} (<https://orcid.org/0000-0002-4743-9577>), Zuzana Ferencova³ (<https://orcid.org/0000-0002-0915-0916>), Víctor González-García^{1,2} (<https://orcid.org/0000-0002-8949-7943>), Borja Jiménez-Alfaro^{1,2} (<https://orcid.org/0000-0001-6601-9597>)

¹ Biodiversity Research Institute (IMIB), University of Oviedo - CSIC - Principality of Asturias, Mieres, Asturias, Spain; ² Department of Organismal and Systems Biology, University of Oviedo, Oviedo/Uviéu, Asturias, Spain; ³ Jardín Botánico Atlántico, Gijón/Xixón, Spain

Correspondence: Eduardo Fernández-Pascual, Instituto Mixto de Investigación en Biodiversidad, Campus de Mieres, Edificio de Investigación, 5ª planta, c/ Gonzalo Gutiérrez Quirós s/n, E-33600 Mieres, Spain. Email: fernandezpeduardo@uniovi.es. Telephone: +34985104781.

Author contributions

Eduardo Fernández-Pascual: Conceptualization; Methodology; Investigation; Data Curation; Formal Analysis; Visualization; Writing – Original Draft Preparation; Writing – Review & Editing. Zuzana Ferencova: Conceptualization; Methodology; Investigation; Data Curation; Writing – Review & Editing. Víctor González-García: Investigation; Visualization; Writing – Review & Editing; Borja Jiménez-Alfaro: Methodology; Writing – Review & Editing.

Acknowledgements

María García Fernández, Sara Gutiérrez Cruz and Abelardo Sigüenza Solís helped with the field surveys. Thanks to AllGenetics & Biology SL (A Coruña, Spain) for DNA metabarcoding analysis and to INEA Laboratory (Valladolid, Spain) for soil physicochemical analysis.

Declaration of interest statement

We have nothing to declare.

Funding

The project is supported by the Biodiversity Foundation of the Ministry for the Ecological Transition and the Demographic Challenge (MITECO) of the Government of Spain, within the framework of the Recovery, Transformation and Resilience Plan (PRTR), funded by the European Union - NextGenerationEU. Ayuntamiento de Gijón/Xixón, Jardín Botánico Atlántico (SV-23-GIJON-JBA). Grant “Laboratorio de Vegetación y Biodiversidad” (IDE/2024/000720, Principality of Asturias-Sekuens-EU-FEDER). Víctor González-García was supported by the Government of Asturias (Programa “Severo Ochoa” PA-22-BP21-098).

Data availability statement

Original datasets, R code for analysis and Rmarkdown code for creation of the manuscript can be accessed at Zenodo: [DOI WILL BE INSERTED HERE].

1 Compositional novelty of plant, fungal and bacterial communities across urban habitats

2 Running title: Compositional novelty across urban habitats

3 **Abstract (250 words)**

4 Understanding urban community novelty can help to predict the rewilding potential of vacant
5 urban spaces, facilitating their integration into biodiverse cities. We tested the hypothesis that
6 cities are composed of different degrees of ecological novelty by studying four urban habitats
7 with differing degrees of management and human legacy: park lawns, roadsides, residential
8 vacant lots, and industrial vacant lots. We focused on community compositional novelty, by
9 comparing the plant, fungal and bacterial species composition between urban habitats and
10 two reference pre-urban habitats: forests and meadows. We used a compositional novelty
11 index based on multidimensional ordination, which is straightforward to calculate and only
12 requires species co-occurrence data for urban and reference habitats. As expected, (1) plants
13 displayed the highest novelty and bacteria the lowest; (2) urban communities were markedly
14 different from forests and relatively more similar to meadows; and (3) compositional novelty
15 was highest in industrial lots. Managed park lawns, which we had expected to be highly
16 novel, were relatively close to hay meadows. The lowest novelty was recorded in residential
17 vacant lots, which had biological communities that more closely resembled those of pre-
18 urban habitats. Our results highlight the effect of habitat type as a major driver of urban
19 community composition and novelty. This suggests that city biodiversity can be enhanced by
20 an integrative approach to the urban landscape that favors habitat heterogeneity by passive
21 rewilding of park lawns, non-intervention on residential vacant lots, direct restoration of

1
2
3
4
5 22 industrial lots, and conservation of natural and agricultural habitat remnants as sources of
6
7
8 23 native species.
9

10
11
12 24 **Keywords (7)**
13
14

15 25 Urban biodiversity, vegetation, soil fungi, soil bacteria, DNA metabarcoding, soil pollution,
16
17
18 26 novel ecosystems
19
20
21

22
23 27 **Highlights (3 to 5, 85 words each)**
24

- 25 28 • A compositional novelty index based on multidimensional ordination is
26
27 29 straightforward to calculate and only requires species co-occurrence data for urban
28
29
30 30 and reference pre-urban habitats.
31
32 31 • Novelty was the highest for plant communities and the lowest for soil bacteria.
33
34 32 • Urban communities were markedly different from forest communities and relatively
35
36 33 more like meadow communities.
37
38 34 • Compositional novelty was highest in industrial vacant lots, and lowest in residential
39
40 35 vacant lots.
41
42 36 • Park lawns were relatively close in composition to hay meadows.
43
44
45
46
47
48
49

50 37 **Introduction**
51
52

53 38 Urban development affects landscape composition and related biodiversity patterns
54
55 39 worldwide ([Grimm et al., 2008](#)). Urbanization converts natural and agricultural habitats into
56
57
58 40 a fine-scale mosaic of diverse urban habitats varying in size, fragmentation, past and present
59
60
61
62
63
64
65

land uses, and degrees of human intervention (Pauleit & Breuste, 2011). At the same time, novel urban habitats experience climatic differences from the surrounding landscape, as well as high within-city microclimatic variation (Forman, 2014; Parlow, 2011). Urban soils likewise vary in permeability, compaction, organic matter content and human-concentrated chemicals (Forman, 2014; Sauerwein, 2011).

Urban habitats are colonized by organisms from the regional species pool that are capable of living in urban environments (Johnson et al., 2017). The process of colonization requires species to pass through a series of hierarchical filters (biogeographical, abiotic, biotic and human) resulting in the formation of an urban species pool and the assembly of specialized urban communities (Aronson et al., 2016; Swan et al., 2021). Urbanization promotes specialized ruderal species and traits (Di Giulio et al., 2024; Raposo et al., 2024; Sotillo et al., 2024), and thus urban communities are rich in species that can tolerate urban disturbance, drought and pollution (Forman, 2014). For this reason, the urban biota can be a source of nature-based solutions, such as the bioremediation of degraded soils (Matanzas et al., 2021; Song et al., 2019) and ecosystem design (Klaus & Kiehl, 2021; Perrelet et al., 2024). Urban communities also host a high proportion of the regional biodiversity (Fernández-Pascual et al., 2025; Gentili et al., 2024) and make up a major part of the supply of natural elements in densely populated landscapes (Kowarik, 2018). Proper management of urban communities can turn them into assets with high ecological and social returns (Anderson & Minor, 2017; Fekete et al., 2024; Itescu & Jeschke, 2024).

The diversity of urban communities can be characterized along two axes: naturalness and novelty (Kowarik, 2018). Naturalness refers to the degree of self-organization, i.e., the extent

1
2
3
4
5
6 63 to which community composition and functions are self-assembled or dependent on direct
7
8 64 human inputs (Kowarik, 2018). Novelty refers to the degree of differentiation from the
9
10 65 historical reference communities that existed in the area before urbanization (Teixeira et al.,
11
12 66 2021; Teixeira & Fernandes, 2020). For example, urban communities under direct human
13
14
15 67 management (e.g., park lawns) should have low naturalness and high novelty. When
16
17
18 68 management stops, naturalness increases, and the community can either transition back to a
19
20 69 state resembling the historical reference (e.g., a low novelty hay meadow) or into a novel
21
22 70 assemblage of species (e.g., a high novelty urban grassland with non-native species) (Teixeira
23
24
25 71 et al., 2021; Teixeira & Fernandes, 2020). The fate of a specific urban site will depend on the
26
27 72 interaction between the legacy of human intervention and inherent site characteristics
28
29
30 73 (Cramer et al., 2008). Intense human legacies usually push communities into novel states,
31
32 74 often dominated by non-native and otherwise unwanted taxa (Dunn & Heneghan, 2011;
33
34 75 Forman, 2014), which usually require more intensive and costly actions to restore the land
35
36
37 76 for conservation purposes (Cramer et al., 2008). Understanding the patterns and drivers of
38
39 77 urban community novelty can help to predict the restoration and rewilding potential of vacant
40
41
42 78 urban spaces, facilitating their integration into the management of biodiversity in cities.

43
44
45 79 The goal of this article was to test the hypothesis that urban habitats are comprised of different
46
47 80 degrees of ecological novelty (Teixeira & Fernandes, 2020). We addressed this hypothesis
48
49
50 81 by studying four urban habitats representing different land uses and human legacies, from
51
52 82 managed park lawns to relatively unmanaged strips of land along transportation corridors,
53
54
55 83 physically altered soils in residential vacant lots, and highly contaminated industrial
56
57 84 brownfields. To address community compositional novelty, we compared the plant, fungal

and bacterial species composition between urban habitats and two reference pre-urban habitats: forest remnants and hay meadows. We expected novelty to be the highest for plants, which in many cases are the object of direct management and introduction (Lázaro-Lobo et al., 2024); and lowest for bacteria, which should be less affected than fungi by the usually alkaline urban soils (Forman, 2014). We expected novelty to be higher when urban habitats are compared to natural habitats (forests), and lower when compared to agricultural habitats (meadows). We also expected novelty to be higher in the actively managed habitat (park lawns) and in the unmanaged habitat with the highest human legacy (industrial brownfields). Finally, we expected that compositional novelty would be related to soil physicochemical properties, reflecting the major aspect of human legacy in urban habitats. To test these expectations, we tackled three main research goals: (1) to characterize the variation in soil properties across urban and pre-urban habitats; (2) to characterize the variation in plant, fungal and bacterial communities across urban and pre-urban habitats; and (3) to measure the compositional novelty across habitats and taxonomic groups.

Materials and methods

Study city

Gijón/Xixón is a medium-sized coastal city (c. 270,000 inhabitants; Instituto Nacional de Estadística) located in the Bay of Biscay, within the central Asturias metropolitan area (c. 800,000 inhabitants) of northern Spain. The city belongs to the Cantabrian Mixed Forests ecoregion (Fernández-Pascual et al., 2025; Lázaro-Lobo et al., 2024), at the border between the temperate and Mediterranean climatic zones of Europe. Climate is temperate oceanic with

1
2
3
4
5
6 106 cool summers and wet and mostly frost-free winters (mean annual temperature = 13.5 °C,
7
8 107 annual precipitation = 1062 mm; 1981-2010 data provided by Agencia Estatal de
9
10 108 Meteorología). Most of the city is built on alluvial and estuarine deposits, sand or limestone
11
12 109 bedrock. The potential pre-human vegetation was likely dominated by deciduous mixed
13
14 110 forests, together with riparian forests, wetlands and coastal dunes. The area has a long history
15
16 111 of human habitation dating back to the Cantabrian Upper Paleolithic (Straus, 2005). The city
17
18 112 itself has been inhabited at least since Roman times (Fernández Ochoa & Martínez Díaz,
19
20 113 1987). During the medieval and early modern times, the urban center was limited to the
21
22 114 original walled city, with fishing being the main economic activity. Expansion beyond the
23
24 115 original core started in the 18th and 19th centuries. Exposure to global trade networks and
25
26 116 the warm temperate climate turned the region into a hotspot for biological invasions,
27
28 117 especially from the Americas and Asia (Lázaro-Lobo et al., 2024). In 1852, the city was
29
30 118 connected by railway to regional mining and industrial centers and became a major shipping
31
32 119 port (Gómez-Martín & Cañal-Fernández, 2024). During the following century the city
33
34 120 experienced significant industrial development (e.g., canning, coal, steel, shipbuilding) and
35
36 121 rapid population growth, becoming the most populated city in the Principality of Asturias
37
38 122 (Obeso Muñoz & Fernández García, 2017). Industrial and urban growth brought
39
40 123 environmental problems including loss of natural wetlands and air and watercourse pollution
41
42 124 (Utanda Moreno & Feo Parrondo, 1995). Starting in the 1970s, the city and the metropolitan
43
44 125 area have been affected by post-industrial land-use changes leaving large abandoned
45
46 126 industrial sites (i.e., brownfields) (Gallego et al., 2016; Matanzas et al., 2021).
47
48
49
50
51
52
53
54
55
56

57 *Definition of urban habitats*
58
59
60
61
62
63
64
65

To survey urban biodiversity across habitats, we defined six habitat types (**Figure 1A**) representing different theoretical states of naturalness and novelty (Kowarik, 2018): (1) forest patches < 10 km from the city center, i.e., remnants of pre-urban natural habitats; (2) late mown hay meadows, i.e., remnants of pre-urban agricultural habitats; (3) park lawns, i.e., green spaces created and maintained by humans; (4) roadsides, i.e., relatively unmanaged strips of land alongside roads and train tracks; (5) residential vacant lots, i.e., currently unmanaged land which has suffered some degree of soil physical disturbance and is waiting to be urbanized; and (6) industrial vacant lots, i.e., currently unmanaged land with an intense legacy of soil disturbance and contamination.

Sampling plots

During June 2023, we surveyed the city to establish five sampling plots (4 x 4 m) per each of the six habitat types (n = 30 plots). The same plot size was maintained in the forest patches for comparability. The plots were distributed across the city (**Figure 1B**) and represent the variation of vegetation types found within each habitat type. In one plot per habitat (**Figure 1B**), we buried a datalogger (M-Log5W, GeoPrecision, Ettlingen, Germany; accuracy: +/- 0.1 °C (at 0 °C), resolution: 0.01 °C) at a depth of 5 cm below the upper layer of the soil. Dataloggers recorded temperature hourly for one year (1 Jan to 31 Dec 2024), after which we downloaded their records to describe the thermal environment of the city and the habitats.

(A) Habitat types



(B) Distribution of plots across the study area

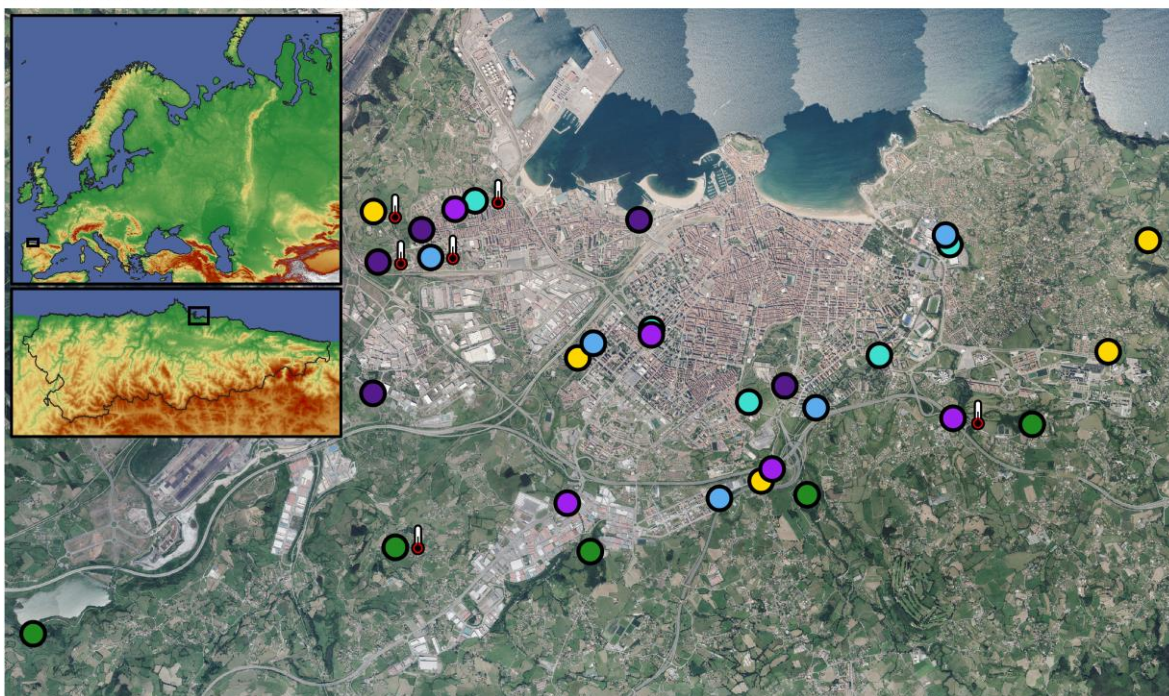


Figure 1: Pre-urban and urban habitats included in this study. (A) Representative pictures of each habitat, indicating their level of naturalness (i.e., degree of ecosystem self-organization) and novelty (i.e., degree of divergence from pre-human reference ecosystems). (B) Location of the sampling plots within the city of Gijón/Xixón, Asturias, Spain, Western Europe. Each plot (dots) is colored according to habitat type. A thermometer to the right of the sampling plot indicates that a soil temperature datalogger was installed there.

Soil physicochemical sampling

From each of the 30 plots, we collected 500 g of soil to analyse soil physicochemical properties. We took the samples from 5 points in each plot (center + 4 cardinal points), down to a depth of 10 cm. We removed stones and other debris, sieved the soils in a 5 mm mesh and homogenized the samples in a plastic bag. Samples were analyzed at INEA Laboratory (Valladolid, Spain), following the standard methodology of the Spanish Ministry of Agriculture (Vallejo Acevedo, 1994). Soil analysis included pH, texture, bulk density, electrical conductivity, organic matter content, total N, C/N ratio, active limestone, total carbonates, assimilable P, K, Ca, Mg, Na and heavy metals (Zn, Cr, Cu, Cd, Hg, Pb, Ni).

Plant, fungal and bacterial diversity sampling

In each plot, we recorded all the spontaneous vascular plant species, as well as the cover (%) of each species in the plot. Plant species names follow Euro+Med (Euro+Med, 2006). Additionally, from each of the 30 plots, we collected soil samples for DNA metabarcoding of fungal and bacterial (including Archaea) communities. We performed the sampling in sterile conditions, and always before entering the plot for any of the other tasks. We cleaned

and sterilized all tools with water and ethanol between samples. In each plot, we took samples from the four cardinal points, 0.5 m inwards from the edge, at a depth of 5 cm. Before taking the samples, we removed the vegetation with a sterile tool. Then, we collected the soil samples in a 50 ml Falcon tube, mixed them, homogenized them with a lancet and finally stored the sample in a 2 ml Eppendorf tube which we immediately froze. The frozen tubes were sent to AllGenetics & Biology SL (A Coruña, Spain) for DNA metabarcoding analysis. The DNA analysis included DNA extraction; library preparation with 2 pairs of different primers specific for barcoding (ITS for fungi, and 16S for bacteria); sequencing on an Illumina NovaSeq PE250 platform (assuming an amplicon size < 450 bp); and standard bioinformatic analysis including demultiplexing, quality control and sample preprocessing, inference of Amplicon Sequence Variants (ASVs), taxonomic assignment, number of reads per taxon and generation of rarefaction curves (see **Appendix S1** for extended DNA metabarcoding methodology).

Statistical analysis and compositional novelty index

We performed all analyses using R version 4.3.1 (R Core Team, 2023) and the package *tidyverse* (Wickham et al., 2019) for data manipulation and visualization. To describe soil temperatures as recorded by the dataloggers, we calculated the mean annual temperature, the mean diurnal range and the annual range. To assess the variability in soil physicochemical properties, we conducted a Principal Component Analysis (PCA) as implemented in the R package *FactoMineR* (Lê et al., 2008) and a correlation analysis to detect auto-correlated soil variables. Multivariate analyses of community composition were performed in *vegan* (Oksanen et al., 2022): we used PERMANOVA to test differences between habitat types in

1
2
3
4
5
6 190 their soil variables and their composition of plant species, fungal ASVs and bacterial ASVs;
7
8 191 Non-metric Multi-Dimensional Scaling (NMDS, see details below) ordination to assess
9
10 192 variation in taxonomic composition of plants (for comparison with fungal and bacterial
11
12 193 communities, we used presence/absence of plant species, instead of cover), fungi
13
14 194 (presence/absence of ASVs) and bacteria (presence/absence of ASVs); and environmental
15
16 195 fitting to test the correlation between community ordination axes and soil physicochemical
17
18 196 properties.
19
20
21
22

23 197 To calculate a compositional novelty index, we used the NMDS multidimensional distance
24
25 198 from each urban plot (i.e., park, roadside, residential or industrial) to the centroid of the two
26
27 199 reference pre-urban habitats (i.e., forests and meadows). We calculated compositional
28
29 200 novelty separately for plant, fungal and bacterial communities. Because NMDS employs an
30
31 201 iterative algorithm to seek optimal solutions, the results may vary across different
32
33 202 computational runs, leading to inconsistencies in ordination outcomes. Therefore, we also
34
35 203 considered an alternative ordination method based on eigenvalue decomposition (Principal
36
37 204 Component Analysis PCA with Hellinger transformation). Choosing NMDS or PCA did not
38
39 205 affect in any way the final output and interpretation of the analyses, and therefore we decided
40
41 206 to use NMDS for the final presentation of results, since it makes less assumptions about data
42
43 207 and is therefore a more flexible technique that can be applied to a wider variety of datasets.
44
45 208 We computed NMDS using the Bray-Curtis distance. When using NMDS for assessing
46
47 209 variation in taxonomic composition, we fitted the analysis to 2 dimensions, since the goal
48
49 210 was visualization of the biplot. In this case, the best solution was achieved after 20 runs for
50
51 211 plants (stress = 0.15), 20 runs for fungi (stress = 0.12) and 20 runs for bacteria (stress = 0.09).
52
53
54
55
56
57
58
59
60
61
62
63
64
65

When using NMDS for calculating novelty, we fitted the analysis to 4 dimensions, to allow for a better fit. In this case, the best solution was achieved after 20 runs for plants (stress = 0.08), 20 runs for fungi (stress = 0.07) and 20 runs for bacteria (stress = 0.04). The plot-centroid distances (i.e., the novelty index) were calculated by summing the distances in each of the four dimensions.

After calculating the novelty indices, we tested the effect of taxonomic group (plant, fungi and bacteria), pre-urban reference habitat (forest or meadow), urban habitat (park, roadside, residential or industrial) and soil physicochemical variables (after reducing their dimensionality by ordination analysis, see results for more details) on compositional novelty, by fitting a Linear Mixed Model with the package *nlme* (Pinheiro et al., 2020). Total sample size in the model was 120 (5 plots x 4 urban habitats x 2 reference habitats x 3 taxonomic groups). Because each sampling plot was included in the model six times (2 reference habitats x 3 taxonomic groups), the model included plot as a random factor. We checked and confirmed model assumptions using the package *performance* (Lüdtke et al., 2021).

Results

Soil properties across habitats

Soil temperature records (**Figure 2A**) indicated that forest soil was relatively buffered in its thermal range of variation, with a mean annual temperature of 13.9 °C, a mean diurnal range of 1.8 °C and an annual range of 10.8 °C. Meadow and urban plots had more thermal oscillation, with a mean annual temperature between 12.4 °C (roadsides) and 16.8 °C

(meadows), a mean diurnal range between 3.8 °C (roadsides) and 4.6 °C (residential lots), and an annual range between 16.5 °C (residential lots) and 19.5 °C (meadows).

The PCA ordination of the soil physicochemical properties (**Appendix S2**) indicated that the first axis of variation, explaining 23% of the variance, ordered soils according to the concentration of heavy metals. The variables with the highest contribution to this axis (> 10%) were the soil contents in Pb, Cu, Zn, Cd and Cr. This first axis did not separate urban from pre-urban habitats: for example, while some industrial lots had a very high concentration of Pb and other metals, the median Pb value of the industrial habitat was below the median value of forests (**Figure 2B; Appendix S2**).

The second PCA axis (**Appendix S2**) explained 21% of the variance and ordered soils according to their compaction and water retention capacities, with the highest contributing variables (> 10%) being organic matter content, bulk density, N concentration and pH. This axis separated soils according to their habitat: for example, forests had high organic matter and N content, while industrial vacant lots had higher bulk density and pH (**Figure 2B; Appendix S2**).

The third PCA axis (**Appendix S2**) explained 10% of the variability and ordered soils according to their content in Mg, with the highest contributing variables (>10%) being Mg content, Mg/K ratio and conductivity. In this case, like for the first axis, there was no clear separation between habitats, with industrial sites having some of the highest and lowest values (**Figure 2B; Appendix S2**).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

252 The analysis showed strong correlations within the groups of variables contributing to the
253 first, second and third PCA axes (**Appendix S2**). For further analyses we kept these three
254 axes as explanatory variables. For simplicity, we will refer to each axis by the name of its
255 most explanatory variable (PC1 = Pb; PC2 = organic matter (OM); PC3 = Mg).

256 As previously indicated by PCA, organic matter was the only soil property with a clear
257 pattern of variation by habitat: a decrease from pre-urban to urban habitats (**Figure 2B**).

258 PERMANOVA supported that there was not a significant difference between habitats in their
259 soil physicochemical properties (100,000 permutations, $R^2 = 0.19$, $F = 1.15$, $p = 0.31$).

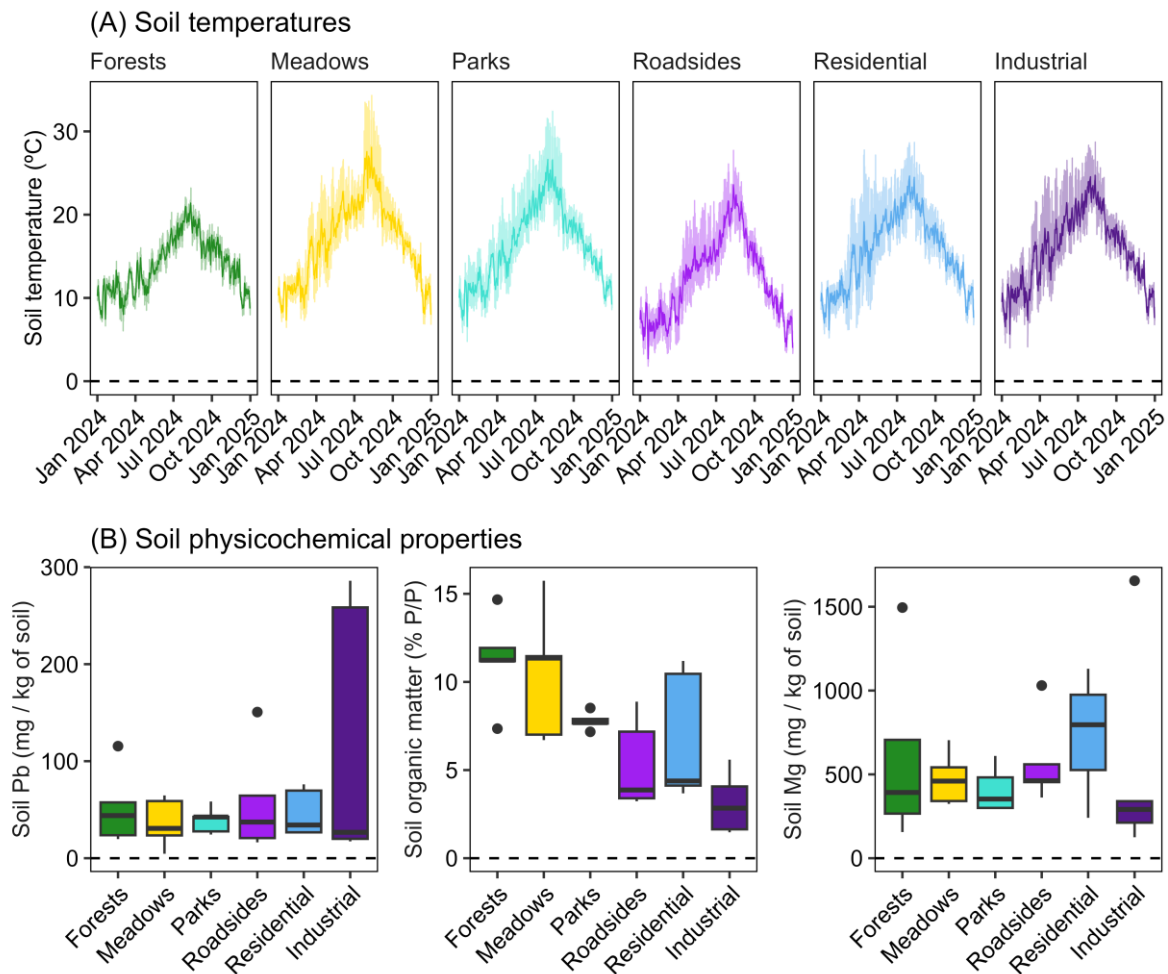


Figure 2: Soil properties across habitats. (A) Temperature recorded every hour in one sampling plot per habitat from 1 Jan 2024 to 31 Dec 2024, indicating the hourly records (shaded colors) and the daily averages (full colors). (B) Soil physicochemical properties. Values are shown for the three most explanatory and uncorrelated variables: soil Pb content, soil organic matter content, and soil Mg content.

Plant, fungal and bacterial communities across habitats

The survey of spontaneous plant diversity yielded 216 species, with the average richness per plot being 26 (min = 10, max = 38). Of these species, 48% were found only in urban habitats

(parks, roadsides, residential lots and industrial lots), 27% occurred only in pre-urban habitats (forests and meadows) and 25% were shared by both urban and pre-urban habitats (**Fig3A**). On average, the habitat with the highest richness per plot was industrial lots (29 species), followed by residential lots (27), roadsides (24), meadows (23), parks (20) and forests (18). The most frequent species were *Holcus lanatus* (22 plots), *Plantago lanceolata* (20), *Helminthotheca echinoides* (16), *Dactylis glomerata* (15) and *Lotus corniculatus* (14). PERMANOVA supported that the plant species composition was significantly different across habitats (100,000 permutations, $R^2 = 0.42$, $F = 3.48$, $p = < 0.001$). The NMDS ordination of the plant species composition (**Fig. 3D**) indicated that the plots belonging to the same habitat were relatively grouped together. The major gradient in vegetation composition along the first axis was a separation between forest plots and the other habitats. Environmental fitting of the Pb, organic matter and Mg vectors onto the ordination indicated that only Pb had a significant correlation ($R^2 = 0.29$, $p = 0.008$) with the composition of the fungal communities. High values of Pb (and other heavy metals) were associated with the plant communities from industrial vacant lots and roadsides (**Fig. 3D**).

The DNA metabarcoding of soil fungal communities resulted in 4,689 amplicon sequence variants (ASVs). Of these, 46% were only found in urban habitats (parks, roadsides, residential lots and industrial lots), 36% occurred only in pre-urban habitats (forests and meadows) and 18% were shared by both urban and pre-urban habitats (**Fig3B**). The average number of ASVs per plot was 340 (min = 174, max = 587), with the habitat averages decreasing from residential vacant lots (415) to forests (393), meadows (336), parks (323), industrial vacant lots (300) and roadsides (276). The majority of ASVs were assigned to

Ascomycota (56%), followed by Basidiomycota (24%), Rozellomycota (10%) and Glomeromycota (4%). Forest habitats had the highest proportion of Basidiomycota (25% vs. < 17% in the other habitats) and the lowest proportion of Ascomycota (57% vs. > 64%). The proportion of Rozellomycota was similar across habitats (from 6% in meadows to 11% in parks). The proportion of Glomeromycota varied between 5% in meadows to 0.5% in forests. PERMANOVA performed on the presence/absence of ASVs indicated significant differences in composition between habitats (100,000 permutations, $R^2 = 0.33$, $F = 2.39$, $p = < 0.001$). The NMDS ordination of the fungal ASV composition (**Fig. 3E**) behaved similarly to the ordination of plant communities, with plots tending to group by habitat, and the major compositional gradient reflecting the separation between forest plots and the rest. Environmental fitting of the Pb, organic matter and Mg vectors onto the ordination indicated that only organic matter had a significant correlation ($R^2 = 0.46$, $p < 0.001$) with the composition of the fungal communities. Low organic matter content was associated with the fungal communities from industrial vacant lots and roadsides (**Fig. 3E**).

For bacterial communities, DNA metabarcoding yielded 16,306 ASVs. Of these, 44% were found only in urban habitats (parks, roadsides, residential lots and industrial lots), 31% occurred only in pre-urban habitats (forests and meadows) and 25% were shared by both urban and pre-urban habitats (**Fig3C**). The average number of ASVs per plot was 1769 (min = 1478, max = 2074), with the habitat averages decreasing from roadsides (1848) to industrial lots (1794), residential lots (1792), meadows (1775), parks (1706) and forests (1697). The majority of ASVs were assigned to Proteobacteria (22%), Planctomycetota (15%), Actinobacteriota (15%) and Acidobacteriota (10%). Compared to other habitats, forests had

313 higher proportions of Proteobacteria and Acidobacteriota, a lower proportion of
 314 Actinobacteriota, and a similar proportion of Planctomycetota. PERMANOVA performed
 315 on the presence/absence of ASVs indicated significant differences in composition between
 316 habitats (100,000 permutations, $R^2 = 0.32$, $F = 2.51$, $p = < 0.001$). The NMDS ordination of
 317 the bacterial ASV composition (**Fig. 3F**) resulted in less grouping by habitat than for plants
 318 and fungi, but still reflected a main compositional gradient from forests to industrial vacant
 319 plots. As in the case of the fungal communities, environmental fitting of the Pb, organic
 320 matter and Mg vectors onto the ordination indicated that only organic matter content had a
 321 significant correlation ($R^2 = 0.39$, $p = 0.001$) with the composition of the bacterial
 322 communities. Low organic matter content correlated with the communities from industrial
 323 vacant lots and roadsides (**Fig. 3F**).

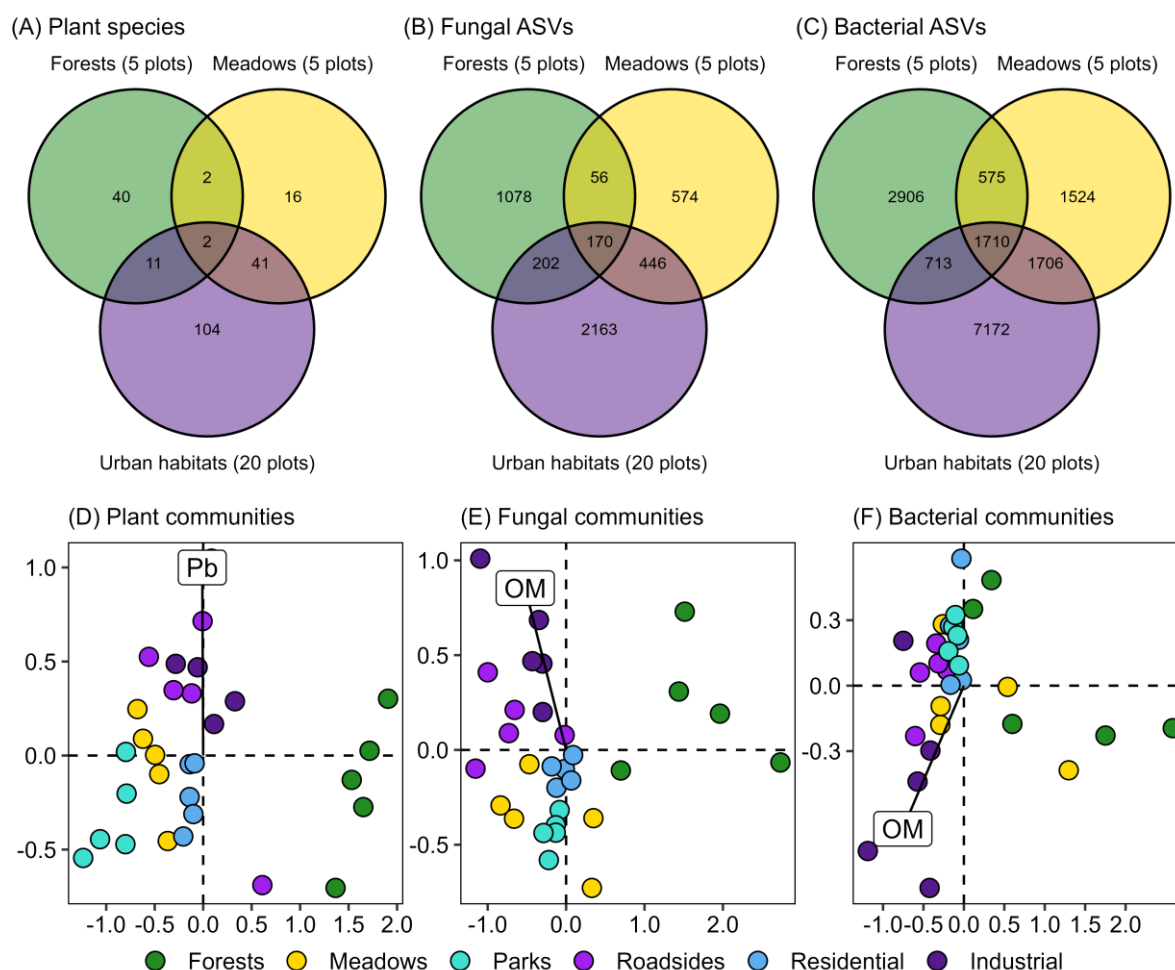


Figure 3: Plant, fungal and bacterial diversity across habitats. Top panels show Venn plots for the shared numbers of (A) plant species, (B) fungal ASVs and (C) bacterial ASVs between pre-urban (forests, meadows) and urban (parks, roadsides, residential lots and industrial lots) habitats. Bottom panels show biplots of community composition ordination produced by Non-metric Multi-Dimensional Scaling (NMDS). The NMDS were conducted for (D) the plant survey (by species presence/absence), (E) the soil fungal survey (by ASV presence/absence) and (F) the soil bacterial survey (by ASV presence/absence). Colors

1
2
3
4
5 332 *indicate the habitat. In the biplots, arrows and labels indicate soil variables with a significant*
6
7 333 *correlation with the composition of the communities.*
8
9

10 334 *Compositional novelty across habitats and taxonomic groups*
11
12
13

14 335 We used the NMDS multidimensional compositional distance (calculated from the
15
16 336 ordinations in **Fig. 3D-F**) from each of the urban plots (i.e., park, roadside, residential or
17
18 337 industrial) to the centroid of the two reference pre-urban habitats (i.e., forests and meadows)
19
20 338 to calculate a compositional novelty index (**Fig. 4**). Novelty was significantly different
21
22 339 between taxonomic groups ($F = 40.67$, $p < 0.001$), being higher for plants (2.37 ± 0.34 S.E.)
23
24 340 than for fungi (2.15 ± 0.40 S.E.) and bacteria (1.56 ± 0.30 S.E.). Novelty was also
25
26 341 significantly different depending on the pre-urban habitat that was used as a reference ($F =$
27
28 342 227.99 , $p < 0.001$), being higher compared to forests (2.60 ± 0.28 S.E.) than to meadows
29
30 343 (1.46 ± 0.28 S.E.). Nonetheless, for each taxonomic group, there was a high positive
31
32 344 correlation between the novelty compared against forests and meadows ($r = 0.5$ for plants;
33
34 345 0.7 for fungi; 0.9 for bacteria). Different urban habitats also differed significantly in their
35
36 346 novelty ($F = 10.06$, $p = 0.001$), being higher in industrial vacant lots (2.39 ± 0.32 S.E.) than
37
38 347 in roadsides (2.17 ± 0.36 S.E.), parks (1.88 ± 0.42 S.E.) and residential vacant lots ($1.66 \pm$
39
40 348 0.34 S.E.). Of the soil physicochemical properties (i.e., the Pb, organic matter, and Mg axes),
41
42 349 only Mg had a marginally significant positive effect on compositional novelty ($F = 5.63$, $p =$
43
44 350 0.03).
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

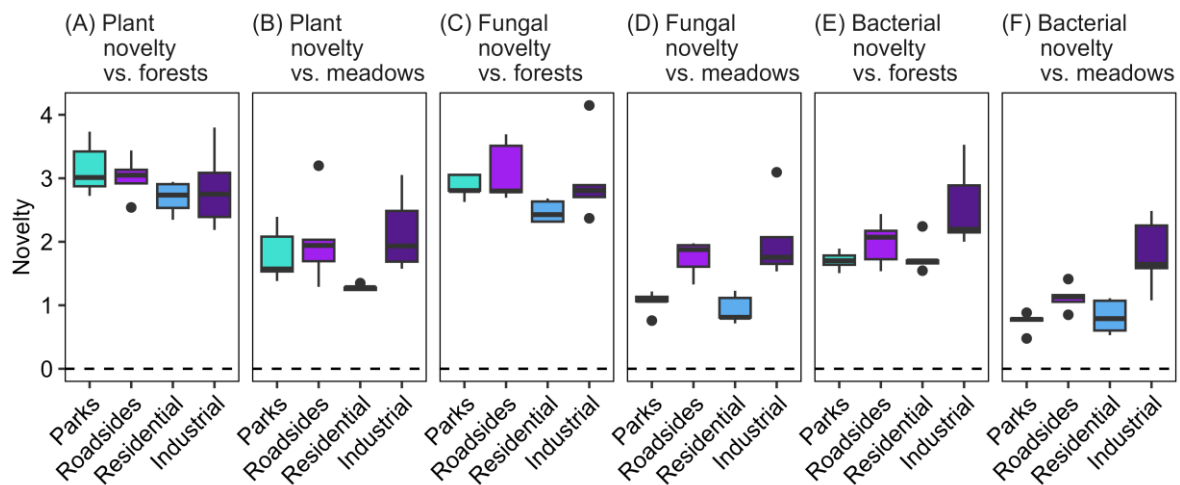


Figure 4: Compositional novelty for plants, fungi and bacteria. Compositional novelty was calculated as the NMDS multidimensional distance from each urban plot to the centroid of two reference pre-urban habitats (forests, panels A-C-E; and meadows, panels B-D-F). Novelty was calculated separately for plants (panels A-B), fungi (panels C-D) and bacteria (panels E-F).

Discussion

This study shows the compositional novelty of biological communities across urban habitats when they are compared with two reference pre-urban habitats: forest remnants and agricultural hay meadows. As expected, (1) novelty was the highest for plant communities and the lowest for soil bacteria; (2) urban communities were markedly different from forest communities and relatively more similar to meadow communities; and (3) the degree of compositional novelty was the highest among industrial vacant lots. However, managed park lawns, which we had expected to be highly novel, were relatively close in composition to hay meadows. Among urban habitats, the residential vacant lots had the lowest novelty, with

biological communities that more closely resembled those of the reference pre-urban habitats.

Contrasting diversity patterns in plant-bacterial vs. fungal communities

Our survey found 216 vascular plant species growing in the study plots, representing 9 % of the regional flora (Fernández Prieto et al., 2014). The highest plant species richness was recorded in vacant industrial and residential plots, while forests displayed the lowest. It must be considered that vacant lots, especially those of industrial origin, had a high number of non-native species, including five that are classified as highly impactful invasives in the ecoregion: *Buddleja davidii*, *Cortaderia selloana*, *Dittrichia viscosa*, *Paspalum dilatatum* and *Sporobolus indicus* (Lázaro-Lobo et al., 2024). Still, vacant lots had more species than forests even considering only native plants. Contrary to plants, fungi had higher richness in our forest plots than in the roadsides or industrial vacant lots, with forests being especially rich in Basidiomycota, as found in other studies (Hui et al., 2017; Marczyklo et al., 2021). This agrees with previous reports of fungal communities decreasing with urbanization (Abrego et al., 2020). One potential explanation for this decrease is the tendency of urban soils to be alkaline, favoring bacteria over fungi (Delgado-Baquerizo et al., 2021; Forman, 2014). Indeed, in our study, organic matter content (highly and inversely correlated with pH) was the soil variable that had an effect on the composition of fungal and bacterial communities; and bacterial richness was higher in urban habitats versus forests (i.e., the opposite to fungi).

Relatively low novelty in parks lawns and residential vacant lots

We used a compositional novelty index based on multidimensional ordination, which is straightforward to calculate and only requires data on species co-occurrence for urban and reference habitats. As such, this index can help to characterize and compare novelty across urban habitats, which is a major research goal for novel ecosystem research (Teixeira et al., 2021; Teixeira & Fernandes, 2020). Contrary to our expectations, managed park lawns showed a relatively low novelty for all three taxonomic groups when compared to agricultural meadows. This indicates that park lawns have a high potential for low-cost urban rewilding policies, such as low-frequency and late mowing, which could convert lawns into urban grasslands rich in native biota and resembling traditional hay meadows. Urban grasslands are able to hold a high portion of the plant diversity found in their rural counterparts (Swacha et al., 2024), especially when seminatural habitat remnants are available in the neighboring landscape to serve as a source of species (Gros et al., 2023). We also found that residential vacant lots have a relatively low level of novelty, including a high richness of fungi and native plants. These temporarily unmanaged sites are therefore a highly valuable asset to integrate biodiversity in cities (Anderson & Minor, 2017; Kowarik, 2018). Additionally, identifying synanthropic and native species able to thrive in these vacant lots can help to find candidate species for urban landscaping that require low levels of watering, fertilization, weeding or mowing (Bretzel et al., 2024; Fekete et al., 2024). Such native and hardy seed mixes are important tools for urban greening, given the increasing awareness of the importance of using locally sourced seed for restoration and rewilding (Hancock et al., 2023).

Conclusions

Our results highlight the effect of habitat type as a major driver of urban community composition and compositional novelty, supporting the findings of previous studies (Gill et al., 2020; Hui et al., 2017; Marczylo et al., 2021). The high impact of habitat types on shaping urban communities and their compositional novelty may be related to the fact that anthropogenic vegetation is highly responsive to habitat-specific disturbance regimes (Fernández-Pascual et al., 2025), while soil microbial communities are in turn highly conditioned by plant inputs (Hui et al., 2017). These results suggest that city biodiversity can be enhanced by an integrative approach to the urban landscape that favors habitat heterogeneity by passive rewilding of managed park lawns, non-intervention on residential vacant lots, direct restoration of industrial vacant lots, and conservation of natural and agricultural habitat remnants as sources of native species (Dylewski et al., 2023; Fekete et al., 2024; Labadessa & Ancillotto, 2023). In this way, the potential of urban habitats can be mobilized so they continue to support ecosystem functioning in anthropogenic city landscapes (Fan et al., 2023; Torija et al., 2025; Weiskopf et al., 2024).

References

Abrego, N., Crosier, B., Somervuo, P., Ivanova, N., Abrahamyan, A., Abdi, A., Hämäläinen, K., Junninen, K., Maunula, M., Purhonen, J., & Ovaskainen, O. (2020). Fungal communities decline with urbanization—more in air than in soil. *The ISME Journal*, 14(11), 2806–2815. <https://doi.org/10.1038/s41396-020-0732-1>

- 427 Anderson, E. C., & Minor, E. S. (2017). Vacant lots: an underexplored resource for
428 ecological and social benefits in cities. *Urban Forestry & Urban Greening*, 21, 146–152.
429 <https://doi.org/10.1016/j.ufug.2016.11.015>
- 430 Aronson, M. F. J., Nilon, C. H., Lepczyk, C. A., Parker, T. S., Warren, P. S., Cilliers, S. S.,
431 Goddard, M. A., Hahs, A. K., Herzog, C., Katti, M., La Sorte, F. A., Williams, N. S. G.,
432 & Zipperer, W. (2016). Hierarchical filters determine community assembly of urban
433 species pools. *Ecology*, 97(11), 2952–2963. <https://doi.org/10.1002/ecy.1535>
- 434 Bretzel, F., Vannucchi, F., Pezzarossa, B., Paraskevopoulou, A., & Romano, D. (2024).
435 Establishing wildflower meadows in anthropogenic landscapes. *Frontiers in Horticulture*,
436 2, 1248785. <https://doi.org/10.3389/fhort.2023.1248785>
- 437 Cramer, V. A., Hobbs, R. J., & Standish, R. J. (2008). What’s new about old fields? Land
438 abandonment and ecosystem assembly. *Trends in Ecology & Evolution*, 23(2), 104–112.
439 <https://doi.org/10.1016/J.TREE.2007.10.005>
- 440 Delgado-Baquerizo, M., Eldridge, D. J., Liu, Y.-R., Sokoya, B., Wang, J.-T., Hu, H.-W., He,
441 J.-Z., Bastida, F., Moreno, J. L., Bamigboye, A. R., Blanco-Pastor, J. L., Cano-Díaz, C.,
442 Illán, J. G., Makhalanyane, T. P., Siebe, C., Trivedi, P., Zaady, E., Verma, J. P., Wang,
443 L., ... Fierer, N. (2021). Global homogenization of the structure and function in the soil
444 microbiome of urban greenspaces. *Science Advances*, 7(28), eabg5809.
445 <https://doi.org/10.1126/sciadv.abg5809>

- 446 Di Giulio, M., Lososová, Z., Carboni, M., & Ricotta, C. (2024). Measuring plant functional
447 specialization in urban environments with Grime's CSR strategies. *Applied Vegetation*
448 *Science*, 27(3), e12803. <https://doi.org/10.1111/avsc.12803>
- 449 Dunn, C. P., & Heneghan, L. (2011). Composition and Diversity of Urban Vegetation. In J.
450 Niemelä, J. H. Breuste, T. Elmqvist, G. Guntenspergen, P. James, & N. E. McIntyre
451 (Eds.), *Urban Ecology: Patterns, Processes, and Applications* (pp. 103–115). Oxford
452 University Press. <https://doi.org/10.1093/acprof:oso/9780199563562.003.0013>
- 453 Dylewski, Ł., Banaszak-Cibicka, W., Maćkowiak, Ł., & Dyderski, M. K. (2023). How do
454 urbanization and alien species affect the plant taxonomic, functional, and phylogenetic
455 diversity in different types of urban green areas? *Environmental Science and Pollution*
456 *Research*, 30(40), 92390–92403. <https://doi.org/10.1007/s11356-023-28808-y>
- 457 Euro+Med. (2006). *Euro+Med PlantBase - the information resource for Euro-*
458 *Mediterranean plant diversity*. <http://ww2.bgbm.org/EuroPlusMed/>.
- 459 Fan, K., Chu, H., Eldridge, D. J., Gaitan, J. J., Liu, Y.-R., Sokoya, B., Wang, J.-T., Hu, H.-
460 W., He, J.-Z., Sun, W., Cui, H., Alfaro, F. D., Abades, S., Bastida, F., Díaz-López, M.,
461 Bamigboye, A. R., Berdugo, M., Blanco-Pastor, J. L., Grebenc, T., ... Delgado-Baquerizo,
462 M. (2023). Soil biodiversity supports the delivery of multiple ecosystem functions in
463 urban greenspaces. *Nature Ecology & Evolution*, 7(1), 113–126.
464 <https://doi.org/10.1038/s41559-022-01935-4>
- 465 Fekete, R., Valkó, O., Fischer, L. K., Deák, B., & Klaus, V. H. (2024). Ecological restoration
466 and biodiversity-friendly management of urban grasslands – a global review on the current

- state of knowledge. *Journal of Environmental Management*, 368, 122220.
<https://doi.org/10.1016/j.jenvman.2024.122220>
- Fernández Ochoa, C., & Martínez Díaz, B. (1987). Gijón, fortaleza romana en el Cantábrico. *Cuadernos de Prehistoria y Arqueología de La Universidad Autónoma de Madrid*, 13-14, 185–203.
- Fernández Prieto, J. A., Cires, E., Bueno Sánchez, Á., Vázquez, V., & Nava Fernández, H. S. (2014). Catálogo de las plantas vasculares del Principado de Asturias. *Documentos Del Jardín Botánico Atlántico*, 11, 1–355.
- Fernández-Pascual, E., González-García, V., Ivesdal, G., Lázaro-Lobo, A., & Jiménez-Alfaro, B. (2025). Classification and characterization of anthropogenic plant communities in the northwestern Iberian Peninsula. *Applied Vegetation Science*, 28, e70010.
<https://doi.org/10.1111/avsc.70010>
- Forman, R. T. T. (2014). *Urban Ecology: Science of Cities*. Cambridge University Press.
<https://doi.org/10.1017/CBO9781139030472>
- Gallego, J. R., Rodríguez-Valdés, E., Esquinas, N., Fernández-Braña, A., & Afif, E. (2016). Insights into a 20-ha multi-contaminated brownfield megasite: an environmental forensics approach. *Science of The Total Environment*, 563-564, 683–692.
<https://doi.org/10.1016/j.scitotenv.2015.09.153>
- Gentili, R., Quaglini, L. A., Galasso, G., Montagnani, C., Caronni, S., Cardarelli, E., & Citterio, S. (2024). Urban refugia sheltering biodiversity across world cities. *Urban Ecosystems*, 27(1), 219–230. <https://doi.org/10.1007/s11252-023-01432-x>

- 1
2
3
4
5 488 Gill, A. S., Purnell, K., Palmer, M. I., Stein, J., & McGuire, K. L. (2020). Microbial
6
7
8 489 composition and functional diversity differ across urban green infrastructure types.
9
10 490 *Frontiers in Microbiology*, 11, 912. <https://doi.org/10.3389/fmicb.2020.00912>
11
12
13 491 Gómez-Martín, M., & Cañal-Fernández, V. (2024). Industry, literature, and sociability: the
14
15 492 effects of industrialisation of Asturian parishes according to Armando Palacio Valdés.
16
17 493 *Rural History*, 35(2), 342–359. <https://doi.org/10.1017/S0956793324000062>
18
19
20
21 494 Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., & Briggs, J.
22
23 495 M. (2008). Global change and the ecology of cities. *Science*, 319(5864), 756–760.
24
25 496 <https://doi.org/10.1126/science.1150195>
26
27
28
29 497 Gros, C., Bulot, A., Aviron, S., Beaujouan, V., & Daniel, H. (2023). Both management
30
31 498 practices and landscape influence plant communities in urban grasslands. *Frontiers in*
32
33 499 *Ecology and Evolution*, 11, 1151913. <https://doi.org/10.3389/fevo.2023.1151913>
34
35
36
37 500 Hancock, N. M., Encinas-Viso, F., & Broadhurst, L. M. (2023). A documented paradigm
38
39 501 shift in seed sourcing: attitudinal changes to using local native seed for ecological
40
41 502 restoration. *Restoration Ecology*, 31(4), e13845. <https://doi.org/10.1111/rec.13845>
42
43
44
45 503 Hui, N., Jumpponen, A., Francini, G., Kotze, D. J., Liu, X., Romantschuk, M., Strömmer, R.,
46
47 504 & Setälä, H. (2017). Soil microbial communities are shaped by vegetation type and park
48
49 505 age in cities under cold climate. *Environmental Microbiology*, 19(3), 1281–1295.
50
51 506 <https://doi.org/10.1111/1462-2920.13660>
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 507 Itescu, Y., & Jeschke, J. M. (2024). Assessing the conservation value of cemeteries to urban
508 biota worldwide. *Conservation Biology*, 38(6), e14322.
509 <https://doi.org/10.1111/cobi.14322>
- 510 Johnson, A. L., Borowy, D., & Swan, C. M. (2017). Land use history and seed dispersal drive
511 divergent plant community assembly patterns in urban vacant lots. *Journal of Applied
512 Ecology*, 55, 451–460. <https://doi.org/10.1111/1365-2664.12958>
- 513 Klaus, V. H., & Kiehl, K. (2021). A conceptual framework for urban ecological restoration
514 and rehabilitation. *Basic and Applied Ecology*, 52, 82–94.
515 <https://doi.org/10.1016/j.baae.2021.02.010>
- 516 Kowarik, I. (2018). Urban wilderness: supply, demand, and access. *Urban Forestry & Urban
517 Greening*, 29, 336–347. <https://doi.org/10.1016/j.ufug.2017.05.017>
- 518 Labadessa, R., & Ancillotto, L. (2023). Small but irreplaceable: the conservation value of
519 landscape remnants for urban plant diversity. *Journal of Environmental Management*,
520 339, 117907. <https://doi.org/10.1016/j.jenvman.2023.117907>
- 521 Lázaro-Lobo, A., Campos, J. A., Díaz González, T. E., Fernández-Pascual, E., González-
522 García, V., Marchante, H., Romero Buján, M. I., & Jiménez-Alfaro, B. (2024). An
523 ecoregion-based approach to evaluate invasive species pools. *NeoBiota*, 96, 105–128.
524 <https://doi.org/10.3897/neobiota.96.116105>
- 525 Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis.
526 *Journal of Statistical Software*, 25(1), 1–18. <https://doi.org/10.18637/jss.v025.i01>

- 527 Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021).
 528 performance: an R Package for assessment, comparison and testing of statistical models.
 529 *Journal of Open Source Software*, 6(60), 3139. <https://doi.org/10.21105/joss.03139>
- 530 Marczylo, E. L., Macchiarulo, S., & Gant, T. W. (2021). Metabarcoding of soil fungi from
 531 different urban greenspaces around Bournemouth in the UK. *EcoHealth*, 18(3), 315–330.
 532 <https://doi.org/10.1007/s10393-021-01523-1>
- 533 Matanzas, N., Afif, E., Díaz González, T. E., & Gallego, J. R. (2021). Phytoremediation
 534 potential of native herbaceous plant species growing on a paradigmatic brownfield site.
 535 *Water, Air, & Soil Pollution*, 232(7), 290. <https://doi.org/10.1007/s11270-021-05234-9>
- 536 Obeso Muñoz, Í., & Fernández García, F. (2017). Recent urban development in Gijón (Spain).
 537 Historic aerial photography as a tool for sustainability assessment of the process. *Cities*,
 538 67, 1–8. <https://doi.org/10.1016/j.cities.2017.04.009>
- 539 Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R.,
 540 Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B.,
 541 Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., ... Weedon, J. (2022).
 542 *vegan: Community Ecology Package. R package version 2.6-4.* [https://CRAN.R-](https://CRAN.R-project.org/package=vegan)
 543 [project.org/package=vegan](https://CRAN.R-project.org/package=vegan).
- 544 Parlow, E. (2011). Urban Climate. In J. Niemelä, J. H. Breuste, T. Elmqvist, G.
 545 Guntenspergen, P. James, & N. E. McIntyre (Eds.), *Urban Ecology: Patterns, Processes,*
 546 *and Applications* (pp. 31–44). Oxford University Press.
 547 <https://doi.org/10.1093/acprof:oso/9780199563562.003.0005>

- 548 Pauleit, S., & Breuste, J. H. (2011). Land-Use and Surface-Cover as Urban Ecological
549 Indicators. In J. Niemelä, J. H. Breuste, T. Elmqvist, G. Guntenspergen, P. James, & N.
550 E. McIntyre (Eds.), *Urban Ecology: Patterns, Processes, and Applications* (pp. 19–30).
551 Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780199563562.003.0004>
- 552 Perrelet, K., Moretti, M., Dietzel, A., Altermatt, F., & Cook, L. M. (2024). Engineering blue-
553 green infrastructure for and with biodiversity in cities. *Npj Urban Sustainability*, 4(1), 27.
554 <https://doi.org/10.1038/s42949-024-00163-y>
- 555 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & Team, R. C. (2020). *nlme: Linear and*
556 *Nonlinear Mixed Effects Models. R package version 3.1–148.*
- 557 R Core Team. (2023). *R: a language and environment for statistical computing. Version*
558 *4.3.1.* <https://www.r-project.org/>
- 559 Raposo, K. S. P., Damasceno-Junior, G. A., Almeida-Gomes, M., & Araujo, A. C. (2024).
560 The effects of urbanization on functional dispersion of plant reproductive traits in Cerrado
561 fragments. *Urban Ecosystems*, 27(3), 741–755. [https://doi.org/10.1007/s11252-023-](https://doi.org/10.1007/s11252-023-01476-z)
562 [01476-z](https://doi.org/10.1007/s11252-023-01476-z)
- 563 Sauerwein, M. (2011). Urban Soils—Characterization, Pollution, and Relevance in Urban
564 Ecosystems. In J. Niemelä, J. H. Breuste, T. Elmqvist, G. Guntenspergen, P. James, & N.
565 E. McIntyre (Eds.), *Urban Ecology: Patterns, Processes, and Applications* (pp. 45–58).
566 Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780199563562.003.0006>
- 567 Song, Y., Kirkwood, N., Maksimović, Č., Zheng, X., O'Connor, D., Jin, Y., & Hou, D.
568 (2019). Nature based solutions for contaminated land remediation and brownfield

- 569 redevelopment in cities: a review. *Science of The Total Environment*, 663, 568–579.
570 <https://doi.org/10.1016/j.scitotenv.2019.01.347>
- 571 Sotillo, A., Hardion, L., Chanez, E., Fujiki, K., & Muratet, A. (2024). Plant responses to
572 urban gradients: extinction, plasticity, adaptation. *Journal of Ecology*, 112(12), 2861–
573 2875. <https://doi.org/10.1111/1365-2745.14427>
- 574 Straus, L. G. (2005). The Upper Paleolithic of Cantabrian Spain. *Evolutionary Anthropology: Issues, News, and Reviews*, 14(4), 145–158. <https://doi.org/10.1002/evan.20067>
- 576 Swacha, G., Raduła, M. W., Jewtich, S., Kusak, B., & Świerszcz, S. (2024). Varying patterns
577 of taxonomic and functional plant composition and diversity across different types of
578 urban and rural grasslands. *Land Degradation & Development*, 35(16), 4997–5010.
579 <https://doi.org/10.1002/ldr.5273>
- 580 Swan, C. M., Brown, B., Borowy, D., Cavender-Bares, J., Jeliaskov, A., Knapp, S.,
581 Lososová, Z., Padullés Cubino, J., Pavoine, S., Ricotta, C., & Sol, D. (2021). A framework
582 for understanding how biodiversity patterns unfold across multiple spatial scales in urban
583 ecosystems. *Ecosphere*, 12(7), e03650. <https://doi.org/10.1002/ecs2.3650>
- 584 Teixeira, C. P., & Fernandes, C. O. (2020). Novel ecosystems: a review of the concept in
585 non-urban and urban contexts. *Landscape Ecology*, 35(1), 23–39.
586 <https://doi.org/10.1007/s10980-019-00934-4>
- 587 Teixeira, C. P., Fernandes, C. O., Ahern, J., Honrado, J. P., & Farinha-Marques, P. (2021).
588 Urban ecological novelty assessment: implications for urban green infrastructure planning

589 and management. *Science of The Total Environment*, 773, 145121.

590 <https://doi.org/10.1016/j.scitotenv.2021.145121>

591 Torija, M. G., Quintana, J. R., Pino-Bodas, R., & Molina, J. A. (2025). Contribution of

592 ruderal herbaceous vegetation to supporting services in Mediterranean urban greenspaces.

593 *Biodiversity and Conservation*, 34(1), 173–189. <https://doi.org/10.1007/s10531-024->

594 [02964-0](https://doi.org/10.1007/s10531-024-02964-0)

595 Utanda Moreno, L., & Feo Parrondo, F. (1995). Problemática medioambiental en la región

596 central asturiana en la primera mitad del siglo XX: su percepción en las topografías

597 médicas. *Anales de Geografía de La Universidad Complutense*, 15, 759–768.

598 Vallejo Acevedo, J. M. (1994). Análisis de suelos. Métodos químicos. In *Método oficiales*

599 *de análisis. Tomo III*. Ministerio de Agricultura, Pesca y Alimentación. Secretaría General

600 Técnica.

601 Weiskopf, S. R., Lerman, S. B., Isbell, F., & Lyn Morelli, T. (2024). Biodiversity promotes

602 urban ecosystem functioning. *Ecography*, 2024(9), e07366.

603 <https://doi.org/10.1111/ecog.07366>

604 Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund,

605 G., Hayes, A., Henry, L., & Hester, J. (2019). Welcome to the Tidyverse. *Journal of Open*


606 *Source Software*, 4(43), 1686. <https://doi.org/10.21105/joss.01686>

607 **Supplementary**


608 **Appendix S1** Extended methodology for the soil DNA metabarcoding analysis.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

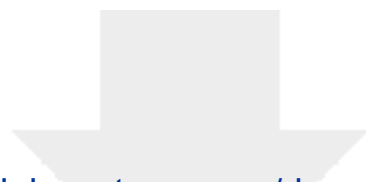
609 **Appendix S2** Principal Component Analysis and correlation analysis of the soil
610 physicochemical properties.



Click here to access/download
Supplementary Material
AppendixS1.pdf



Click here to access/download
Supplementary Material
AppendixS2.pdf



[Click here to access/download](#)

Supporting File
manuscript-track-changes.docx

