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Urban plant ecology and biodiversity: multiple approaches to investigate role, functional traits, responses and conservation of plants in cities

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Un’idea, finché resta un’idea, è soltanto un’astrazione.
Se potessi mangiare un’idea avrei fatto la mia rivoluzione
(Gaber G. “Un’idea”)

Wilderness appealed to those bored or disgusted with man and his works. It not only offered an escape from society but also was an ideal stage for the Romantic individual to exercise the cult that he frequently made of his own soul. The solitude and total freedom of the wilderness created a perfect setting for either melancholy or exultation.
(Nash R., “Wilderness and the American mind”)

It was the masterful and incommunicable wisdom of eternity laughing at the futility of life and the effort of life. It was the Wild.
(London J., “White Fang”)

Rather than love, than money, than fame, give me truth
(Thoreau H.D., “Walden or, Life in the Woods”)

Preface

The work presented in this thesis was carried out in the period from January 2023 to December 2025at the Department of Agricultural Sciences (University of Naples Federico II). Tutor and supervisor was Prof. Giovanna Aronne. A period was spent working with Prof. Peter Glasnović and Prof. Edy Fantinato at the University of Primorska (Department of Biodiversity) and at the Ca' Foscari University of Venice (Department of Environmental Sciences, Informatics and Statistics). The project was funded by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4— Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. In particular, the doctorate was carried out within the activities of spoke 5 (urban environment and health) of the National Biodiversity Future Centre (NBFC), a center dedicated to frontier research, established and funded by the NRRP.

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Introduction

Urbanization is the process through which human populations increasingly concentrate in cities, driving extensive land-use conversion, infrastructure expansion, and profound alterations of ecological dynamics. Since this process is constantly accelerating worldwide, there is an urgent need to reconcile urban growth with biodiversity conservation and to deeply investigate plant ecology in urban ecosystems, as vegetation represents the base component in sustaining biodiversity, maintaining ecosystem functions, and improving human well-being. Expanding over natural habitats, urbanization reshapes landscapes, ecosystems, and threats biodiversity worldwide. Habitat loss and fragmentation, reduction in the quality of air and soil, biotic homogenization, biological invasions and intense human disturbance are well-known outcomes. Collectively, these factors expose plants to multiple interacting pressures that limit their growth, reproduction, survival and alter species richness, community composition, and functional trait diversity. While urban pressures generally challenge species survival, they also create a unique combination of extreme and novel conditions that strongly and rapidly interact with plants, making cities a valuable opportunity for studies on plant reaction and adaptation to specific environmental factors.

From an ecological perspective, urbanization is a complex and multifaceted phenomenon, presenting hostile conditions for most species while simultaneously offering opportunities for others, owing to their heterogeneous structures, diverse habitats, and varied microclimates. Within this dynamic environment, urban plants provide essential ecosystems functions but are also subjected to strong environmental stresses.

Plants are fundamental components of Urban Green Spaces (UGS), being able to mitigate adverse effects of urbanization and to support biodiversity functioning. For example, urban forests can provide suitable habitats for plants, support ecological communities, and promote species movement across the urban matrix. Of course, many years are necessary to verify the long-term ecological effects of planting new urban forest. In this scenario, historic urban forests, being planted many decades ago, can represent important case studies for assessing their long-term contribution to urban biodiversity.

Among the numerous environmental disturbances, plants in cities also must deal with soil deterioration and urban soil substrates, including asphalt and foundry sand. Moreover, UGS often relies on the unsustainable practice of importing topsoil from natural areas to ensure plant growth, raising concerns on natural soil depletion. Considering this, targeted experiments are truly important to identify viable alternatives to the current overexploitation of natural topsoil.

Urban conditions generally exert a negative effect on richness and relative abundance of native plant species. However, high habitat heterogeneity, human intervention and the establishment within regions with naturally high biological diversity, make cities favorable to host rare or threatened species. Nevertheless, it is not totally clear how endangered species are distributed across such heterogeneous environments and large-scale floristic data analysis can help fill this gap.

Given the above, a deep understanding of the risks and opportunities exerted by urban environments on plants requires urban ecology studies to operate across multiple spatial scales, addressing both community-level and species-level dynamics. Such research should integrate field sampling, data analyses, and applied botanical experiments. It should also bring together diverse expertise in a multidisciplinary framework. Collaboration with relevant institutions is also essential to ensure that this knowledge can be translated into practical applications that enable people and nature to coexist harmoniously in urban settings.

The study and research activities carried out during this Ph.D. project followed the approach described above. Specifically, I approached urban plant ecology first by in-depth bibliographic research, which resulted in a review of the role of plants in the city (chapter 1). Next, a field sampling was conducted to investigate the functional traits of plant communities in a city with a historic urban forest (chapter 2). Moreover, an experiment was done to analyze the effect of alternative substrates, typical of the urban environment, on the growth of a single species (chapter 3). Finally, floristic data were collected from several European cities to investigate the conservation role of different urban environments (chapter 4).

To achieve this variety of goals, I collaborated with researchers from the University of Naples Federico II, Department of Agricultural Sciences, and expanded to other scientific institutions, mainly the University of Primorska, the Ca' Foscari University of Venice and Royal Botanic Gardens "Kew". Additionally, I attended the Training school in Biology and Pollination Ecology (Module II) at the University of Coimbra promoted by the COST Action ConservePlants CA18201, during which I have acquired knowledge and methodologies in pollen biology, pollination experiments, plant-pollinator interactions, and nectar dynamics.

Some results obtained during the activities of the Ph.D. period have been presented at two international conferences: the 21st Annual Meeting of the Spanish Group for Floral Ecology (EcoFlor 24) and the X International Plant Science Conference (IPSC) and the XI IPSC.

Main activities and results gathered during my PhD period can be shared in four parts that constitute the chapters of this thesis. Aims and content of each chapter are reported hereby.

Chapter 1 is a review which presents the current state of knowledge on plants and urban environments. It started considering what is known about urban plants as threatened organisms and therefore underlining the potential of plants both as bioindicators of environmental changes and as mitigators of the same stressors. By focusing on the dual and interconnected role of urban plants, gaps in current research and opportunities for future investigations were identified and discussed. The article has been submitted for publication in Urban Ecosystems.

Chapter 2 presents evidence on the role of The Royal Park of Portici (an urban forest planted three centuries ago) as critical refuge for species less adapted to urbanization. The study investigated the role of the Park in supporting urban plant diversity, focusing on the functional traits of species that use the Park as a refuge versus those that disperse into the surrounding urban matrix. The Royal Park of Portici was used as an ideal study case for investigating the contribution of an ancient urban forest to biodiversity, being a unique example of a long-established forest within a Mediterranean city. Specifically, it was underlined how urban forests can serve as critical refugia for species and as species sources for nearby green spaces. The study was performed in collaboration with the Ca' Foscari University of Venice, where I learned how to design and execute the analysis. Results of the work were published in Journal of Urban Ecology (Rummo et al. 2025).

Chapter 3 refers to an experiment which assessed the effects of asphalt and two types of sands frequently used in pavement construction, namely standard sand (SS) and recycled sand (RS), on the germination and early development of *Borago officinalis*. The aim was to investigate the possibility of reusing these materials as alternative substrates for urban green infrastructure, focusing on problems related to landfill pressure, and the environmental costs of natural soil exploitation. The article reporting this study has been published in Urban Ecosystems (Rummo et al. 2026).

Chapter 4 presents a conservation-based study that focuses on urban environmental heterogeneity and on the opportunities that cities can offer for conservation of IUCN threatened species. Specific habitat types within the European cities were investigated by adopting two habitat classifications. The aim was to determine whether climatic or demographic factors or habitat-level differences within cities play a stronger role in shaping the distribution of species of conservation concern and which of the different types of urban habitat favors their occurrence. The study was performed in collaboration with the

University of Primorska, the Ca' Foscari University of Venice and Royal Botanic Gardens "Kew", where I acquired knowledge and methodologies on urban ecology and on plant conservation. The manuscript is being finalized for submission for publication in Current Plant Biology.

Finally, in *Chapter 5* some general comments and considerations are given.

In addition, an Appendix outlooking the activities conducted during the Ph.D. program is also reported.

Chapter 1

Plants in the City: from fragile organisms requiring protection to key resources improving citizen well-being - Review

1.1. Abstract

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1.2. Urbanization and urban plants

1.2.1. Introduction

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1.2.2. Literature search

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1.4. Urban plants as bioindicators: turning weaknesses into monitoring strength

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1.5. Urban plants as mitigators and sources of human well-being

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1.6. Current gaps and recommendations for future research

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Chapter 2

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Research article

Plant traits in a 300-year-old urban forest: insights for plant conservation in compact cities

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Plant traits in a 300-year-old urban forest: insights for plant conservation in compact cities

2.1. Abstract

As urbanization accelerates worldwide, reconciling urban growth with biodiversity conservation has become increasingly urgent. Urban forests offer a promising solution by supporting biodiversity and mitigating the negative impacts of urbanization. Historic urban forests, such as the Royal Park of Portici (Naples, Italy), founded in 1738, offer a unique opportunity to assess the long-term contribution of these green spaces to urban biodiversity. In the present study we investigated the role of the Royal Park in supporting urban plant diversity, focusing on the functional traits of species that use the Royal Park as a refuge versus those that disperse into the surrounding urban matrix. We recorded vegetative, pollination and dispersal traits and tested if specific traits were associated with species found exclusively in the Royal Park or in both the Royal Park and urban matrix. The results showed that annual plants with generalist pollination and animal-mediated dispersal strategies thrive in the urban matrix, while perennial species with specialized pollination are restricted to less disturbed areas such as the Royal Park. Urban forests can serve as critical refugia for species and as species sources for nearby green spaces. To maximize their ecological function, efforts should prioritize improving connectivity, reducing environmental stress, and minimizing human disturbance, particularly for species with specific ecological requirements.

2.2. Introduction

As urbanization is currently the fastest-growing form of land use worldwide (Lin and Fuller 2013), there is an increasing need for sustainable urban planning based on a sound ecological knowledge of urban biodiversity, which is essential for maintaining ecosystem functions and improving human well-being (Sandifer et al. 2015, Taylor and Hochuli 2015, Reyes-Riveros et al. 2021, Pinto et al. 2022). Across large urban areas, some are often defined as compact, namely a type of densely built-up area that leads to intensification of space use, higher residential density, centralization of services and scarcity of urban green spaces (hereafter UGS; Sushinsky et al. 2013, Balzan et al. 2021), which mostly include spaces to promote public use (e.g. public parks and urban forests) or small remnants of marginal land (e.g. traffic island flower beds). In compact cities, the ecological impacts of urbanization are locally intense and lead to the complete loss and fragmentation of natural and semi-natural habitats (Parris 2016) as they are replaced by impervious surfaces. In compact cities spaces for plant and animal species are usually very small, isolated and interspersed with an urban matrix of unsuitable habitats and physical barriers (built-up and paved surfaces; Melero et al. 2020). These act as filters and hinder the spread of species by reducing landscape connectivity (Sushinsky et al. 2013). Additionally, the few remaining UGS of the compact cities are also exposed to high levels of environmental stress and human disturbance (Davies et al. 2008, Jenks and Jones 2010). Among the environmental stressors and human disturbances associated with compact urban development, high heat retention has been shown to affect species through a warmer climate (urban heat island effect, Deilami et al. 2018). UGS tend to be drier, more nutrient-rich, and more alkaline due to the deposition of nutrients from the air (de Barros Ruas et al. 2022). They also have higher levels of localized disturbance due to high management intensity and human trampling (Liu et al. 2023). The unique environmental conditions that prevail in urban areas lead to a reduction in species richness and changes in their relative abundance (Knapp et al. 2017, Oliveira et al. 2020) which in turn can trigger changes in biotic interactions that lead to further species loss (Olden 2006, Baiser et al. 2012). Nevertheless, UGS serve as refugia for species and facilitate ecological connectivity by enabling the movement of species through fragmented landscapes (Zhao et al. 2022, Lorenzato et al. 2024, Berger et al. 2024). In addition, UGS provide diverse Nature's Contributions to People (NCPs; Mattana et al. 2022) that are critical to human well-being by mitigating the effects of urban heat, reducing noise pollution, improving air quality and carbon sequestration and increasing aesthetic values, all of which contribute to healthier and more sustainable living (Godefroid and Koedam 2007, Lin and Fuller 2013, Evans et al. 2017, Threlfall et al. 2017, Filazzola et al. 2019). For all these reasons, UGS are increasingly recognized as a form of investment in Nature-Based Solutions (NBS) to address pressing global challenges such as biodiversity loss and the climate crisis (Nesshöver et al. 2017) and to meet human needs.

Among UGS, urban forests are advocated as NBS to improve urban biodiversity and mitigate the negative impacts of urbanization on human well-being (Threlfall et al. 2017, Filazzola et al. 2019). The concept of urban forest was first introduced in North America in 1894 (Coke 1894) and transferred to Europe in the 1980s (Konijnendijk et al. 2006). While the forested landscapes of earlier centuries often had utilitarian or aesthetic functions, today's urban forests are purposefully planned and managed to achieve specific ecological and social objectives. In the European context, this includes the integration of trees and wooded areas in different urban spaces such as parks, streets, private gardens and marginal land, to enhance biodiversity, provide ecosystem services and promote human well-being. Among the characteristics of urban forests, their age has been shown to play a crucial role in their current biodiversity and benefits to society (Nagendra and Gopal 2011). It has been shown that the older the urban forests are, the greater the biodiversity supported and thus the ecosystem services provided (Ma et al. 2023). In this context, historic urban forests can provide an opportunity to assess their long-term contribution to urban biodiversity. Unfortunately, there are few examples of historic urban forests that still exist today (Johnson and Handel 2016, Milecka et al. 2019).

The Royal Park of Portici, in southern Italy, on the coast of the Gulf of Naples, is one of them. The Royal Park was founded in 1738 and was originally intended for leisure and hunting and was located on the edge of the urban area. Over time, however, the Royal Park was integrated into the urban area as the city expanded, so that today it is considered part of the urban area and has evolved from a private recreational and hunting forest into a public space. Portici is a compact Mediterranean city that is closely linked to the large conurbation of Naples and has a high population density. The Royal Park of Portici is therefore a unique example of a long-established forest within a compact Mediterranean city, making it an ideal case study for investigating the contribution of an ancient urban forest to biodiversity.

In light of these considerations, the aim of this study was to evaluate the contribution of the Royal Park to biodiversity by analysing its role as a refuge for plant species and as a possible source of plant species for the UGS scattered in the urban matrix. We focused on plant species because they are the fundamental component of green spaces, as they determine their structural characteristics and are the basis for the conservation of animal species in the urban environment (Andrade et al. 2023). Nowadays, studies on plant species in urban areas are mainly assessed from a functional perspective to investigate either conservation (e.g. Hostetler et al. 2011) or effectiveness in providing ecosystem services (e.g. Andrade et al. 2023). Indeed, the analysis of functional traits has been shown to be better suited to capture species response to urbanization, while taxonomic diversity may mask general patterns of ecological strategies (Dolan et al. 2017, de Barros Ruas et al. 2022). In this study, we specifically compared the functional traits of plants exclusive to the Royal Park with those of species that live both outside and inside the Park, not only to determine the contribution of an ancient urban forest to plant diversity, but also to determine the functional identity of species that use the Royal Park as a refuge and those that can disperse in the UGS scattered in the built environment. In addition, this study aims to provide insights that support the achievement of several UN Sustainable Development Goals (SDGs) by advancing knowledge and practical solutions, particularly for Good Health and Well-being (SDG 3), Sustainable Cities and Communities (SDG 11), Climate Action (SDG 13) and Life on Land (SDG 15).

2.3. Materials and methods

2.3.1. Study sites

Portici is a municipality of Naples (southern Italy) which extends over an area of 4.60 km2 (ISTAT 2012) from the Tyrrhenian Sea (Gulf of Naples) to the south-western slope of the Somma- Vesuvius volcano (90 metres above sea level). It is characterized by a Mediterranean climate (summer period of drought which lasts from late May to August). The warm season runs from June to September with average temperatures over 30°C in July and August. Average annual temperature of the last 16 years (2006– 21) has been 17.2°C and average annual precipitation has been 847mm/year. The rainiest month is November (average rainfall: 102mm) and average of consecutive number of days without rain during the year is 19 (ISTAT 2023). With 55,765 inhabitants (ISTAT 2012) and 12,110 inhabitants/km^2, it is currently the second most densely populated municipality in Italy. The high population density is reflected in a very compact urban development, in which green spaces account for <2% of the area of the city. The most important green space of the city is the Royal Park of Portici, which extends upwards from the sea and is surrounded by built-up and paved surfaces. It is a historic example of an urban forest, as in 1738 the King of Naples, Charles of Bourbon, and his wife Maria Amelia decided to create it to enjoy the beauty of the natural landscape around their residence. Since the forest was intended to harbour natural vegetation, particularly for hunting, a large holm oak wood was planted by transporting the soil layer and by bringing in *Quercus ilex* L. plants from Sicily (Stinca and Motti 2009; Fig. 1a).

The Royal Park is currently divided into two parts: The upper part (36 ha, called 'Gussone Park'), managed by the University of Naples Federico II, and the lower part (almost 10 ha), managed by the local administration (Fig. 1b). It is dominated by a mixed Mediterranean holm oak forest (*Q. ilex*) with *Fraxinus ornus* L. and *Celtis australis* L. and a rich shrub layer characterised by *Sambucus nigra* L., *Laurus nobilis* L., *Rhamnus alaternus* L. and *Cytistus villosus* Pourr., as well as several clearings with herbaceous vegetation. The level of disturbance is very low, and management is minimal (mowing and tillage are rare) and mainly focussed on research activities.

In addition to the Royal Park, there are several other UGS in Portici, including public parks and private gardens. However, they are very small (on average <0.2 km^2) and isolated in the urban matrix (average distance 400 m). These sites share common characteristics, such as the dominance of the herbaceous layer (although trees are often present) and a high level of anthropogenic disturbance in the form of management (frequent mowing) and trampling.

Figure 1. Historic image showing the Royal Park in the first phase of its construction (a; 'The slopes of Vesuvius from Portici with a view of the Bourbon Palace', 1783–1784, by Giovan Battista Lusieri) and contemporary photograph of the Royal Park of Portici and the surrounding city (b; Google Earth).

2.3.2. Data collection

At the beginning of 2023, we surveyed all UGS of Portici and identified those that were either free or accessible on request. In total, there were 16 out of 19 spaces that could be surveyed, in addition to the Royal Park (Fig. 2).

An inventory of spontaneously occurring angiosperm species was drawn up for each site as part of three surveys carried out at each site between March and September 2023 to take into account species that develop at different times of the year. Pteridophytes and gymnosperms were excluded due to their rarity. Species nomenclature follows Pignatti et al. (2017) and subspecies were considered at the species level.

For each species recorded, we collected vegetative, pollination and dispersal traits (Table 1), considering two vegetative traits (i.e. life cycle, perennating organs), six pollination traits (i.e. pollination strategy, floral symmetry, floral size, floral tube length, inflorescence type, flower colour), and three dispersal traits (i.e. dispersal strategy, fruit size, seed size). The traits were collected from different sources (i.e. Kühn et al. 2004. Perez-Harguindeguy et al. 2013, Stefanaki et al. 2015, Pignatti et al. 2017; Table 1). The list of plant species, their occurrence in the Royal Park and in UGS in the surrounding urban matrix as well as their traits can be found in Supporting Data S1.

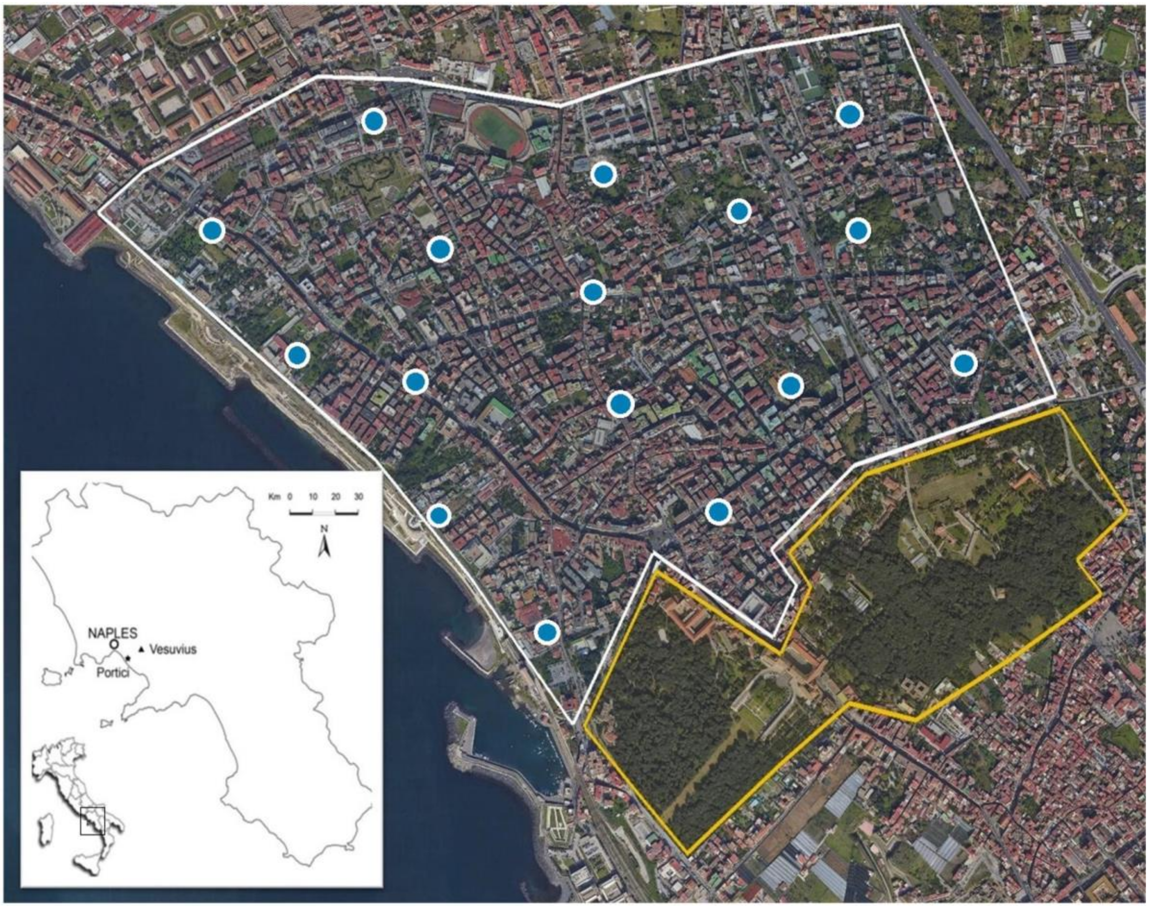

Figure 2. Map of the study site. The Royal Park of Portici is outlined in yellow, while the other surveyed UGS in the urban matrix are represented by blue dots.

2.3.3. Data analysis

To investigate whether different plant traits were associated with species occurring exclusively in the Royal Park or with species occurring in both the Royal Park and in the UGS in the urban matrix, we performed a Pearson's Chi-square test of independence with simulated p-value (based on 9,999 randomizations) for each plant trait with Bonferroni-adjusted post hoc tests in case of the significance of the Chi-square tests (function chisq posthoc.test, chisq.posthoc.test R package; Beasley and Schumacker 1995, Agresti 2007). Considering that the Chi-Square test of independence is used to determine whether there is a significant association between two nominal (categorical) variables (McHugh 2013), we compared the frequency of each trait type and the two species groups, without performing intra-group comparisons. All statistical analyses were performed using R (R Core Team 2024).

Table 1. Traits used to characterize the plant species found in the study site.[a]

Trait category	Trait type	Traits levels	Description	References
Vegetative traits	Life cycle	Annual/Biennial	Plant dies at the end of its first growing season after producing	Raunkiaer 1934, Ellenberg and Müller-Dombois 1967, Whittaker 1975, Klotz *et al.* 2002, Perez-Harguindeguy *et al.* 2013, Pignatti *et al.* 2017
		Perennial	The individual survives for at least three growing seasons.	
		Hidden	Perennating tissue under the ground surface (e.g. bulbs, bulbils)	
	Perennating organs	Basal	Perennating tissue on the ground surface (0–2 cm)	
		Exposed	Perennating tissue above the surface	
		Seeds	Seeds as perennating tissue	
Pollination traits	Pollination strategy	Entomophily	Plants pollinated by insects	
		Anemophily	Plants pollenated by wind	
		Mixed strategy	–	
	Floral symmetry	Radial	Several axes of symmetry	
		Bilateral	One ax of symmetry	
	Floral size	Small	<2 cm	
		Medium	2–10 cm	
		Large	>10 cm	Petanidou 1991, Klotz *et al.* 2002, Petanidou and Lamborn 2005, Stefanaki *et al.* 2015, Pignatti *et al.* 2017, pers. obs.
	Tube length	Short	<4 mm	
		Medium	4–10 mm	
		Long	>10 mm	
	Inflorescence type	Isolated flower	Any type of inflorescence with up to five concurrently open flowers	
		Spherical inflor.	Aggregations of flowers arising on a flat or spherical surface of the inflorescence (heads, umbrellas, corymbs)	
		Cylindrical inflor.	Aggregation of flowers arising along a cylindrical surface of the inflorescence (spikes, racemes, panicles)	
	Flower colour	White	Dominant colour of the corolla (for each single colour variable)	
		Blue		
		Red		
		Yellow		
		Green		
		Double coulored		
Dispersal traits	Dispersal strategy	Anemo/Autochory	Seed dispersed by wind or without external vectors	
		Epizoochory	Seed transported and released involuntarily on the animal's body	
		Endozoochory	Seeds dispersed by animals through ingestion and defecation	Klotz *et al.* 2002, Pignatti *et al.* 2017, pers. obs.
	Fruit size	Small	<5 mm	
		Medium	5–20 mm	
		Large	>20 mm	
	Seed size	Small	<2 mm	
		Medium	2–10 mm	
		Large	>10 mm	

[a] The size classes of seeds were selected after a field survey to determine the minimum, maximum and average sizes from which we formed the categories. The assigned value denotes the diameter in the case of circular shapes, the length in the case of elongated shapes and the average between length and width when seeds and fruits were irregular or different from the previous ones.

2.4. Results

Overall, the list of recorded taxa included 395 species belonging to 254 genera and 81 families, all living in the Royal Park. Among them, 318 species (belonging to 222 genera and 75 families) occurred only in the Royal Park, while 77 species (belonging to 65 genera and 31 families) occurred both in the Royal Park and the UGS in the urban matrix. We did not consider plant species that occurred exclusively in the UGS in the urban matrix and not in the Royal Park (Supporting Data S1). Among the shared species, *Asteraceae* was the most represented family, followed by *Fabaceae*. In contrast, the species exclusive to the Royal Park were dominated by *Poaceae*, followed by *Fabaceae*, reflecting distinct taxonomic patterns between the two groups.

Among the 11 plant traits analysed, Pearson's Chi-square test of independence (with simulated p-values) showed significant variation in the frequency of occurrence for five traits between the two groups (i.e. species that occur exclusively in the Royal Park or with species that occur both in the Royal Park and in the UGS in the urban matrix): life cycle, perennating organs, floral symmetry, tube length, and dispersal strategy (Table 2).

Table 2. Results of Pearson's Chi-square test of independence with simulated *P*-value (based on 9999 randomizations) to test the association between the frequency of each trait in the two groups of species. Bold indicates significant results.

Trait group	Trait	Chi Square statistic	d.f.	*P*-value
Vegetative traits	Life cycle	7.441	1	**0.006**
	Perennating organs	18.344	3	**<0.001**
Pollination traits	Pollination strategy	0.695	2	0.706
	Floral symmetry	7.636	1	**0.005**
	Floral size	3.928	2	0.143
	Tube length	7.186	2	**0.027**
	Inflorescence type	3.482	2	0.175
	Flower colour	4.765	5	0.445
Dispersal traits	Dispersal strategy	13.209	2	**0.001**
	Fruit size	1.065	2	0.587
	Seed size	3.365	2	0.185

Specifically, species with a perennial life cycle and species with hidden perennating organs were more frequently associated than expected (i.e. the observed frequencies are significantly higher than what would be predicted under the null hypothesis of independence in the Chi-square test) with plant species occurring exclusively in the Royal Park (Table 2; Fig. 3a and b), while annual plant species and plant species producing seeds as perennating organs were more frequently than expected associated with plant species that occur both in the Royal Park and in the UGS in the urban matrix (Table 2; Fig. 3a and b). For pollination traits, plant species with flowers exhibiting bilateral symmetry and a long corolla tube were associated more frequently than expected with plant species occurring exclusively in the Royal Park (Table 2; Fig. 3c and d), while plant species with flowers exhibiting radial symmetry were associated more frequently than expected with plant species that occur in both the Royal Park and the UGS in the urban matrix (Table 2; Fig. 3c). Finally, for dispersal strategies, epizoochory was associated more frequently than expected with plant species that occur both in the Royal Park and in the UGS in the urban matrix (Table 2; Fig. 3e).

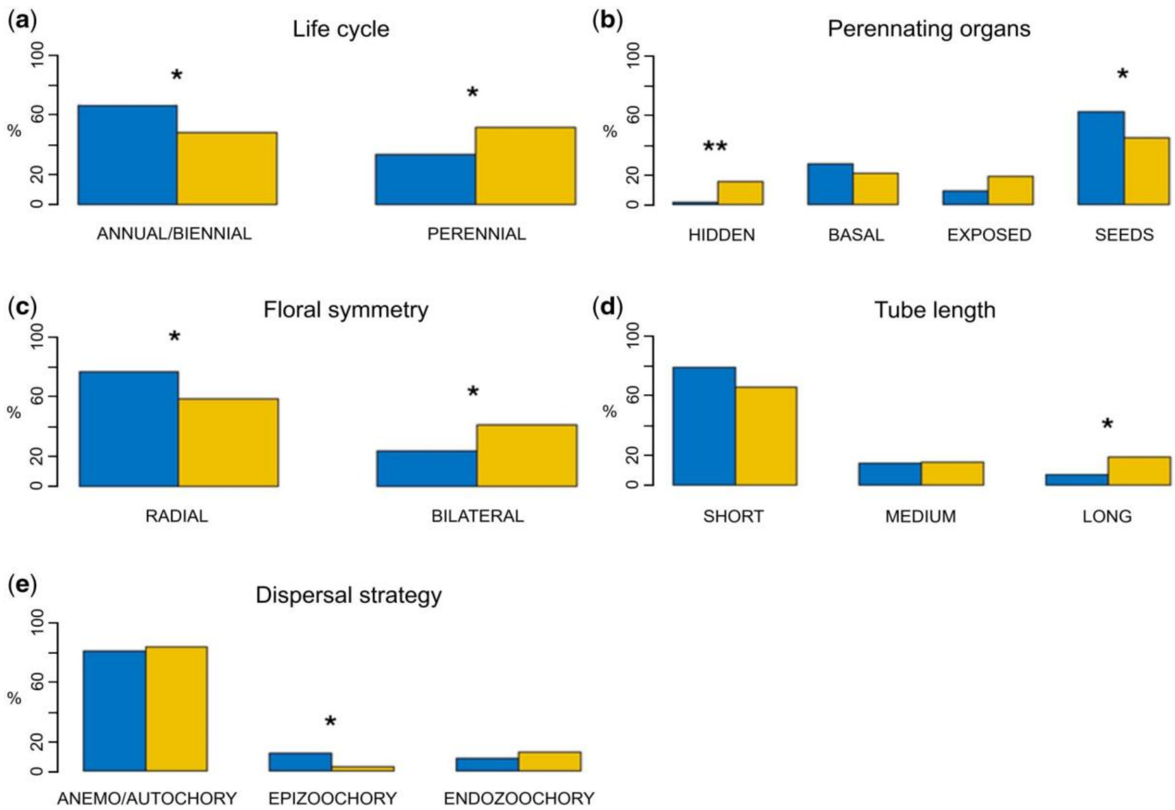

Figure 3. Proportion of plant species per trait within each of the two analysed groups. Trait types displayed are: (a) life cycle, (b) perennating organs, (c) floral symmetry, (d) tube length, and (e) dispersal strategy. Yellow bars represent plant species occurring exclusively in the Royal Park, while blue bars represent plant species that occurred both in the Royal Park and in the UGS in the urban matrix. Significant associations are marked with an asterisk for a P-value <.05 and with two asterisks for a P-value <.01.

Among the plant species present both in the Royal Park and in the UGS in the urban matrix, *Daucus carota* L., *Galium aparine* L., *Torilis arvensis* (Huds.) Link, *Tribulus terrestris* L., and *Urospermum picroides* (L.) Scop. ex F.W. Schmidt exhibited a combination of plant traits associated with plants that can disperse in the urban matrix and establish in UGS, i.e. annual plants with seeds as perennating organs, with flowers showing radial symmetry and a short corolla tube, and epizoochory as a dispersal strategy. Conversely, among plant species exclusive to the Royal Park and showing the combination of plant traits less adapted to the urban environment (i.e. perennials with hidden perennating organs and flowers with bilateral symmetry and long corolla tube) were *Anacamptis coriophora* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *A. morio* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *Arum italicum* Mill., *Epipactis microphylla* (Ehrh.) Sw., *Limodorum abortivum* (L.) Sw., *Spiranthes spiralis* (L.) Chevall and *Serapias lingua* L.

2.5. Discussion

The differences in plant functional traits between species exclusive to the Royal Park and those also occurring in the UGS in the urban matrix indicate a strong ecological segregation driven by urbanization. The two groups differed not only in species composition but also in functional strategies, indicating different ecological filters operating in the two environments. Only 19.5% of the total species pool was shared between the Royal Park and the UGS in the urban matrix, and these shared species mostly exhibited traits indicative of ecological generalization, such as annual or biennial life cycles, zoochorous dispersal mechanisms and generalist pollination systems. In contrast, species restricted to the Royal Park were mainly characterized by perennial life cycles, hidden perennating organs (e.g. rhizomes, bulbs), and specialized pollination strategies, reflecting adaptation to more stable and less disturbed conditions. The observed trait patterns support the interpretation that urbanization acts as a selective force that promotes the persistence of species adapted to disturbance, while filtering out those that require long-term habitat continuity.

UGS in the urban matrix are generally characterized by a high level of human disturbance, which can occur regularly in the form of management (Paudel and States 2023) and/or short-term and occasional trampling (Pescott and Stewart 2014). Human disturbance represents a filtering factor that can select species according to their functional traits (Khan et al. 2023). Annual species with high growth rates have been shown to perform better under human disturbance as they can complete their life cycle between disturbance events. In addition, in the Mediterranean climate most of the precipitation falls in winter and the summers have little or no rainfall. Vegetation regularly experiences both seasonal and inter-annual droughts, which are exacerbated by impervious urban surfaces (Miller et al. 2022). Drought resistance strategies in plants include the annual life cycle, because species spend the drought period as seed rather than as adult organisms. For annual plants that rely on animal pollination, ensuring pollinator visitation is therefore crucial. In highly disturbed urban areas, this can be best achieved by species with flowers having a high degree of generalization for different pollinator species and guilds. Such generalization is often revealed by the floral morphology, where open disc flowers are accessible to a wide range of pollinators (Westerkamp and Classen-Bockhoff 2007), consequently increasing the likelihood of pollination events. In urban environments, this high degree of generalization is even more important as both plant and pollinator species are selectively filtered based on their life history traits, resulting in less predictable pollination visits (Theodorou et al. 2020, Gathof et al. 2022).

Interestingly, among dispersal strategies, epizoochory (i.e. involuntary dispersal mediated by animals via the body surface) was significantly associated with plant species that can disperse in the urban matrix and establish in UGS. This is in some ways surprising, as the dispersal processes of animal-dependent plants are thought to be disproportionately affected by urbanization, as many animals avoid cities or restrict their movements within the urban environment (Gelmi-Candusso and Hämäläinen 2019). However, this dispersal strategy also includes human-mediated dispersal, which is an important mechanism in urban environments (Czarnecka et al. 2013). In comparison with the urban matrix, the Royal Park is relatively little visited by humans. Surely human-mediated seed dispersal is a ubiquitous phenomenon in cities (Bullock et al. 2018) and, according to our results, perhaps the most important factor for the connectivity of green spaces in the urban environment.

Conversely, plant species that are exclusive to Royal Park have functional traits that are associated with stable environmental conditions. These exclusive species are mainly perennial plants with hidden perennating organs (e.g. bulbs). Although such functional type of plants can also thrive in highly disturbed habitats (Del Vecchio et al. 2016), they are particularly common in old forests such as those in the Royal Park (Wulf 2003, Jabs-Sobocińska et al. 2022). In our study, their widespread absence in the UGS in the urban matrix is probably due to their limited dispersal ability and low seed bank persistence (Jabs-Sobocińska et al. 2022), which prevents their return to newly established UGS. In addition, plant species exclusive to the Royal Park showed more than expected flowers with bilateral symmetry and/or a very deep corolla (e.g. tubular or bell-shaped flowers). Complex floral traits, such as bilateral symmetry and long corolla tubes, often correspond to specialized pollination strategies. These specialized plants usually require very evolved pollinators that can handle flowers with complex morphology, such as bumblebees, which are among the species most affected by urbanization (Theodorou et al. 2020). Their absence may therefore result in specialized plants being unable to maintain their populations in UGS exposed to high levels of environmental stress and human disturbance, ultimately highlighting potential indirect effects of urbanization on the pool of plant species that can thrive in UGS.

Functional diversity of plants is associated with the provision of more ecosystem services and thus with a better quality of life for citizens (Diaz et al. 2007). Species with functional traits that are better adapted to urbanization, such as annual plants with generalist pollination and human-mediated dispersal, should be favoured in the creation of self-sustaining UGS, because they are less affected by urbanization. On the other hand, species with functional traits less adapted to urbanization might need to be supported to improve the functional diversity of plants in the urban environment. To improve the functional diversity of plants in compact cities, environmental conditions must be created that meet the physiological and ecological requirements of plant species that are more vulnerable to environmental stress and human disturbance associated with the urban environment. This could be achieved through the planting of urban forests where environmental stress and human disturbance should be minimised (such as the Royal Park of Portici).

Urban forests are an effective solution for conserving biodiversity in compact cities and mitigating the effects of dense urban development (Threlfall et al. 2017). Our findings suggest that historic parks can serve as refugia for species of conservation concern. In Portici, 318 of the 395 species identified occurred exclusively in the Royal Park, including rare or endangered species (Stinca and Motti 2009), such as *Serapias lingua* L., *Allium neapolitanum* Cirillo, *A. roseum* L., *A. triquetrum* L., *Ampelodesmos mauritanicus* (Poir.) T.Durand & Schinz, and *Bellevalia romana* (L.) Sweet., which are included in national Red Lists (Glasnović et al. 2024, Lončarević et al. 2024). However, the limited space and poor ecological connectivity with the urban matrix limit the role of the Royal Park in promoting biodiversity in disturbed urban areas. Less than 20% of the species in the Royal Park were shared with other UGS, mostly species that are widespread and have traits typical of species found in other compact cities (Celesti-Grapow and Blasi 1998, de Barros Ruas et al. 2022), reflecting the global trend towards biotic homogenization in cities (McKinney 2006, Delgado-Baquerizo et al. 2021, Lokatis and Jeschke 2022).

In conclusion, urban forests can serve not only as refugia for species with specific ecological needs, but also as a source of species for surrounding UGS in the urban matrix. However, to maximize their ecological function as a source of species, it is important to improve connectivity with the urban matrix and facilitate the dispersal and establishment of species throughout the city. This requires minimizing environmental stressors and human disturbance, especially to reduce the impact on plant species with specific ecological requirements. Maximizing the ecological function of UGS would in turn also improve ecosystem services, especially those related to biodiversity, such as pollination, climate regulation and aesthetic and recreational value. Integrating ecosystem services into urban planning can ultimately make cities more sustainable and support the achievement of the UN SDGs Good Health and Well-being (SDG 3), Sustainable Cities and Communities (SDG 11), Climate Action (SDG 13) and Life on Land (SDG 15).

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Chapter 3

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RESEARCH

Impact of construction-derived substrates on seed germination and early development of *Borago officinalis*: insight for sustainable management of urban green spaces

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Impact of construction-derived substrates on seed germination and early development of *Borago officinalis*: insight for sustainable management of urban green spaces

3.1. Abstract

Urban vegetation is exposed to intense environmental stress and anthropogenic disturbance, which can strongly constrain plant growth and development. Urban soils differ from natural soils in structure and functionality, being characterized by high compaction and elevated levels of pollutants. They frequently contain anthropogenic materials such as construction and demolition waste (CDW) and asphalt residues (AS), which further alter soil features. Due to their abundance and inert nature, the reuse of these materials as alternative substrates for urban green infrastructure warrants experimental investigation. This study assessed the effects of asphalt and two types of sands frequently used in pavement construction, namely standard sand (SS) and recycled sand (RS), on the germination and early development of *Borago officinalis*. Seeds were sown in trays filled with the test substrates, while potting soil (SO) served as control treatment. We monitored seed germination success, seedling emergence, mortality, biomass accumulation, and evaluated morpho-anatomical and physiological traits such as leaf area, shoot and root dry weight, stomatal density, stomatal conductance, and photochemical efficiency. Seedling emergence and survival were significantly reduced in AS, where mortality reached 60% and leaf development was strongly delayed. As expected, SO plants exhibited the highest biomass and physiological performance, with greater transpiration and lower stomatal density, indicating favourable water use efficiency. Overall, our findings suggest that while AS is unsuitable for early plant development, recycled CDW materials may serve as alternative, though suboptimal, components in the construction of urban Technosols. Their reuse could ease landfill pressure, limit the environmental costs of natural soil exploitation, and support key ecosystem services, thus aligning with circular economy goals and fostering more sustainable urban greening.

3.2. Introduction

Urbanization is the fastest-growing form of land use worldwide (Lin and Fuller 2013) and represents a significant anthropogenic disturbance for plants. Globally, urban inhabitants are more than half of the world's population, and this proportion is expected to rise concurrently with global population growth, being predicted to reach 68% in 2050, as most additional world inhabitants will live in urban areas (UNDESA 2018; European commission 2019).

Although there are some examples of virtuous urbanization, cities are often similar across the world, representing an environment constructed almost exclusively for human use (McKinney 2008). Consequently, natural habitats are increasingly replaced by impervious surfaces and anthropogenic structures such as roads, buildings, and pavements (Grimm et al. 2008). This results in numerous environmental challenges, including increased waste and pollution production, loss of rural and natural land to urban sprawl, and biodiversity loss (Kaye et al. 2006; Grimm et al. 2008; Alberti 2015; Guilland et al. 2018). Most pronounced environmental stresses associated with urban environment include habitat fragmentation, soil degradation, high pollution levels, and high heat retention caused by impervious surfaces (Davies et al. 2008; Jenks and Jones 2009; Deilami et al. 2018). All these pose critical threats to vegetation, often creating unsuitable conditions for plant growth (Kowarik 2011; Aronson et al. 2017). For instance, higher temperatures can accelerate seed desiccation and reduce germination rates, negatively impacting early plant development (Tzoulas et al. 2007), while impervious surfaces like asphalt further exacerbate stress by reducing water infiltration, and soil moisture, thus limiting root expansion and nutrient uptake (Mullaney et al. 2015). Moreover, urbanization selects plant species according to their functional traits (Khan et al. 2023), promoting the persistence of species adapted to human disturbance, while filtering out those that require long-term stable ecological conditions (Rummo et al. 2025).

Urbanization also causes soil deterioration, consequently reducing ecosystems services provided by soil within urban green spaces (UGS), such as plant production in urban agriculture and gardening (Guilland et al. 2018). Urban soils frequently display lower quality and greater heterogeneity than their rural or natural counterparts, differing in structure, composition, and functionality (Kaye et al. 2006; Lehmann and Stahr 2007; Lorenz and Lal, 2009; Edmondson et al. 2014). These substrates often lack in essential nutrients like nitrogen, phosphorus, and potassium, and tend to be drier, more anoxic, and more alkaline than soils from natural areas partly due to deposition of airborne nutrients and pollutants (Biasioli et al. 2007; Pouyat et al. 2010; de Barros Ruas et al. 2022).

According to the World Reference Base for Soil Resources, most urban soils are classified as Technosols, characterized by high contents of anthropogenic materials and/or continuous impermeable seals (Lehmann and Stahr 2007; Rossiter 2007). Also, the French pedological system distinguishes five types of anthropized soils, including artificial anthroposols with non-pedological waste materials, and reconstituted anthroposols using transported topsoil from natural areas for vegetation establishment (Guilland et al. 2018). However, being created under the influence of cities activities, urban soils frequently contain construction or demolition waste (CDW), including asphalt (Ujile and Abbey 2022).

UGS, such as gardens and park, increasingly relies on imports of topsoil from natural areas to ensure plants growth. However, importing large quantities of topsoil from agricultural or forest areas is unsustainable and raises concerns over the depletion of natural soil resources and associated carbon emission, especially given urban population growth and land use pressures (Deeb et al. 2020). Furthermore, the mobilization of natural soils for UGS causes erosion and loss of soil productivity in forested and rural landscapes (Rompato et al. 2025). Concurrently, soil sealing related with urbanization growth reduces both the quality and availability of soils in cities (Fabbri et al. 2021).

Additionally, cities generate large volumes of unproductive materials, such as CDW and asphalt residues (AS), the disposal of which incurs high costs for public administrations (Fabbri et al. 2021).

These concerns have led to growing interest in alternative substrates for UGS, including a focus on the use of urban waste materials for plant cultivation, which may represent a sustainable solution aligned with circular economy principles (Séré et al. 2008; Mikajlo et al. 2025).

CDW represent the largest volume of waste products worldwide (Ujile and Abbey 2022). Recycling these materials instead of landfilling can alleviate disposal expenses for public administration (Jia et al. 2024). Recently, research has increasingly focused on reusing urban waste to mitigate these costs (Deeb et al. 2019). Indeed, constructed Technosols (an engineered mixtures of organic and mineral wastes) based on recycled CDW materials (Abbruzzini et al. 2022; Jia et al. 2024), particularly asphalt (Mikajlo et al. 2025; Rompato et al. 2025), excavation waste (Prado et al. 2020), and foundry sands (Dayton et al. 2010; De Koff et al. 2010), might reduce both urban waste disposal problems and the dependence on overexploited natural topsoil for UGS, while supporting multiple soil functions such as plant growth, water infiltration, and biological activity (Hitchmough et al. 2001; Rokia et al. 2014; Nehls et al. 2015; Deeb et al. 2020; Fabbri et al. 2021).

However, incorporating these materials can alter the chemical and physical properties of urban soils (Lorenz and Lal 2009; Pouyat et al. 2010). Asphalt, a ubiquitous component in cities, can raise soil temperatures, reduce water infiltration, restrict root expansion and plant gas exchange, and introduce petroleum-based contaminants potentially toxic to plants and soil organisms (Jones et al. 2012; Ajuru and Upadhi 2016; Cachada et al. 2016; Zhang et al. 2017; Itodo et al. 2018). Such substrates expose seedlings to water deficits, while root penetration may be restricted by high substrate resistance (Craul 1994). To address challenges such as high compaction, bulk density, and low organic matter, several studies have successfully tested compost amendments (De Koff et al. 2010; Coull et al. 2021; Mikajlo et al. 2025). Nevertheless, despite the availability of these unproductive materials in cities, their impact on plant growth and physiology remain underexplored, with only a few studies specifically assessing the effect of asphalt residues. The use of CDW in constructing Technosols has shown potential to provide both physical support and nutrients for various vegetation types (Yilmaz et al. 2018; Pruvost et al. 2020), including urban agriculture (Araujo et al. 2022; Barbillon et al. 2023), and horticulture (Abbruzzini et al. 2022). Both Mikajlo et al. (2025) and Rompato et al. (2025) suggest that supplementing soils with AS and compost can be safe and effective, although Ajuru and Upadhi (2016) highlight negative impacts on plant species living in asphalt-polluted environments. To our knowledge, little research has directly compared commonly available construction-derived materials, such as asphalt residues and construction sands, as substrates for plant growth, despite their abundance and relevance in urban waste streams. There is a clear need for targeted experiments that determine how individual components of anthropogenic urban soils affect plant development, particularly during early growth stages when plants are most sensitive to substrate conditions. Addressing this gap could help identify viable alternatives to the current overexploitation of natural topsoil while also contributing to improved urban waste management strategies.

To address this challenge, this study experimentally assesses the impact of common construction-derived waste, namely asphalt, standard and recycled construction sand, on the germination and early development of *Borago officinalis.,* a fast-growing cosmopolitan species, widely employed in urban agriculture, community gardening, and known for its sensitivity to soil properties (Gilani et al. 2007; Motti and Motti 2017; Falcinelli et al. 2024).

We hypothesized that the use of construction-derived substrates might affect the early development of *B. officinalis,* compared to potting soils, with potential differences among the different substrates tested. To evaluate this, we compared the effects of the substrates on seed germination and seedling growth, as well as on morpho-anatomical and physiological traits, with the aim of elucidating how these materials influence plant development and offering insights to promote more sustainable practices in urban environments, aligning with the achievement of several UN Sustainable Development Goals (SDGs).

3.3. Materials and methods

3.3.1. Experimental design and growth conditions

This study aimed to investigate the effects of urban construction-derived materials on the germination and early seedling development of *Borago officinalis*, a cosmopolitan species, widely used for agro-food, herbal medicine and ornamental purposes, which is increasingly employed in urban agriculture, community gardening, and foraging (Gilani et al. 2007; Motti and Motti 2017; Falcinelli et al. 2024). Likewise, *B. officinalis* strongly supports urban pollinator communities, enhancing plant-pollination interactions (Theodorou et al. 2016, 2020). The substrates we tested included asphalt (AS) and two types of sands frequently used in pavement construction, namely standard sand (SS) and recycled sand (RS). These materials reflect typical components of urban soils, while commercial potting soil (SO) was used as a control (Fig. 1).

The AS used in this study derived from road construction waste (Fig. 1A). To remove large debris and ensure uniformity we preliminary sieved the milled asphalt using a metal filter with a 2 mm mesh. The two pavement sands, SS and RS, differed markedly in origin, composition, and physical characteristics (Fig. 1B). SS was a commercially available product, typically sold in pre-packaged bags and employed as a base layer in floor construction. Differently, RS was obtained through the mechanical crushing and grinding of heterogeneous building demolition debris. This type of sand is commonly sourced directly from construction material dumps and is often preferred to SS by construction workers due to its lower cost. RS exhibited a more heterogeneous composition and coarser particle size distribution compared to SS, containing fragments of glass, ceramic tiles, bricks, and concrete. For the purpose of this study, all RS material originated from the same processing facility.

The experiment was carried out from March to April 2025 at the Department of Agricultural Sciences, University of Naples Federico II. The Department is located in Portici, a municipality of Naples and the second most densely populated municipality in Italy (Istat 2023). It was performed in an open greenhouse without environmental control, in order to expose the plants to natural urban-like environmental conditions. Seeds were sown in standard polystyrene nursery trays, each comprising 90 cells with drainage holes (cell volume: ≈80 cm^3). Each tray was subdivided into two halves of 45 cells, with each half representing an independent replicate of a substrate treatment. A total of eight trays were used, corresponding to four replicates per substrate treatment, resulting in 720 seeds in total, 180 per substrate and 45 per replicate. Prior to sowing, each type of substrate was carefully homogenized and used to fill the cells uniformly. Seeds were placed individually in each cell to standardize sowing density and facilitate consistent monitoring of germination and seedling development (Fig. 1).

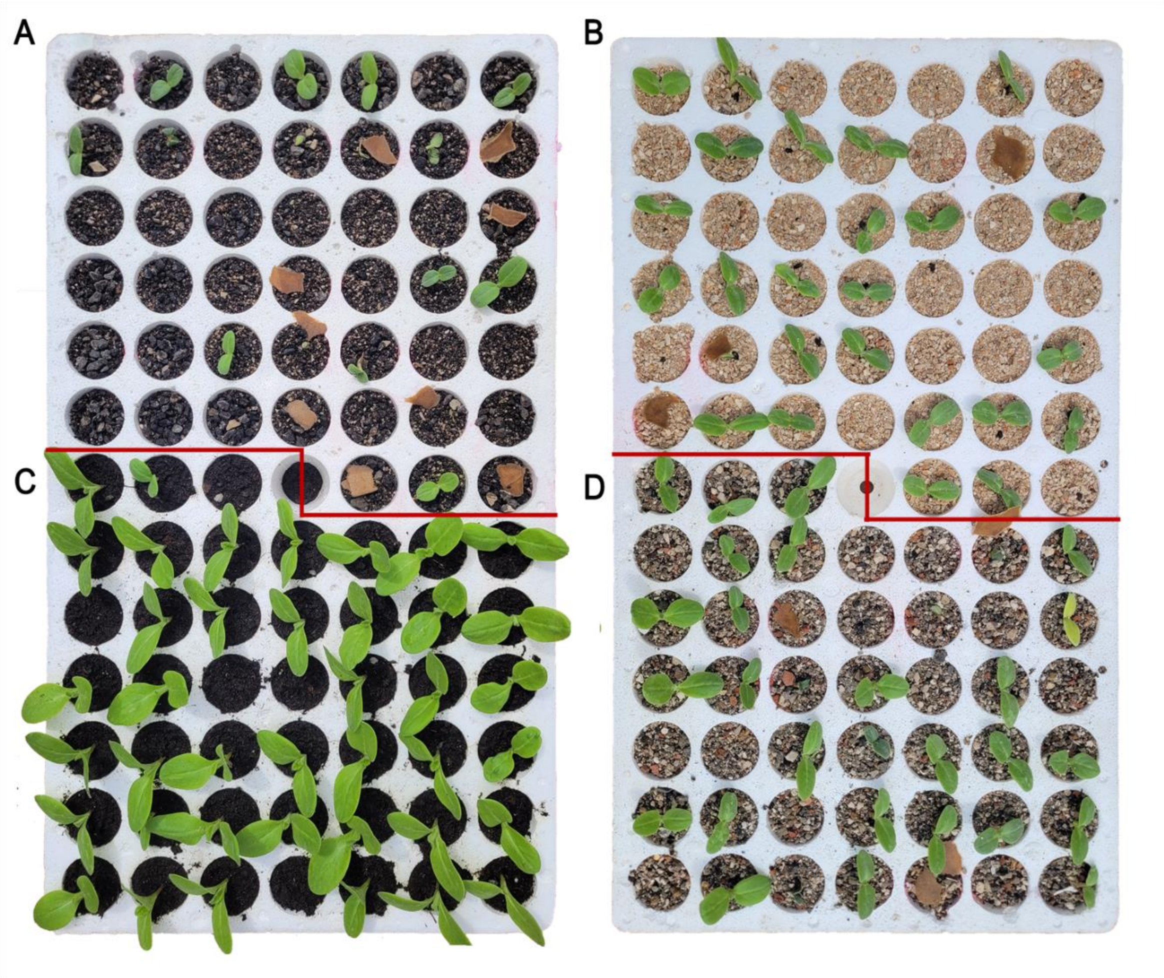

Figure 1. Experimental setup and polystyrene tray used in the study as example of substrate allocation: A) asphalt; B) standard sand; C) potting soil; D) recycled sand.

3.3.2. Growth biometric measurements

Throughout the 43-days experimental period, germination success was assessed through daily observations of seed germination and seedling emergence. Seedling development was monitored by tracking the emergence and morphological progression of cotyledons and true leaves, alongside the recording of mortality rates to detect potential substrate-related stress effects. For each replicate, at each observation day we recorded cumulative counts and converted them into a discrete-time survival format with three columns: time (days after sowing), event (1 = event occurred at that time; 0 = right-censored at that time), and weight (number of individuals). We analysed four endpoints as survival-type outcomes: a)

Ungerminated seeds (%): events correspond to germination; b) Seedlings before cotyledon opening (%): events correspond to first cotyledon opening; c) Seedlings before true leaf emergence (%): events correspond to first true leaf emergence; d) Seedling survival (%): events correspond to mortality.

Biomass assessments included measurements of fresh and dry weights of roots and shoots at two time points: T1 (20 days after sowing; DAS) and T2 (43 DAS). Fresh weights were recorded immediately after harvesting, while dry weights were obtained after oven-drying the samples at 65 °C for 72 hours to constant weight, using a precision analytical balance. Cotyledon and leaf area was measured using high-resolution photographs. Surface area was determined using ImageJ digital image analysis software (National Institutes of Health, USA). Key functional traits were derived to assess resource allocation and leaf structural investment, including the root-to-shoot ratio (R/S), calculated as the ratio of dry root biomass to dry shoot biomass, and leaf mass per area (LMA), determined as the ratio of leaf dry mass to leaf area.

3.3.3. Physiological measurements

To assess plant physiological traits, we measured stomatal conductance and chlorophyll fluorescence parameters at both 20 DAS and 43 DAS on cotyledons and first true-leaves, respectively, using the LI-600 Porometer/Fluorometer (LI-COR Biosciences, Lincoln, NE, USA). For each measurement event, data were collected from 12 cotyledons or 12 leaves per treatment. All measurements were conducted in the morning, between 10:00 and 12:00. The following parameters were measured: maximum quantum efficiency of photosystem II (F_v/F_m), quantum yield of photosystem II (QY), non-photochemical quenching (NPQ), stomatal conductance to CO_2 (g_s), transpiration rate (E), and surface temperature (T).

3.3.4. Stomatal measurements

Stomatal traits were assessed at both 20 DAS and 43 DAS through microscopic observations of cotyledons and true leaves to identify potential anatomical adaptations to substrate conditions (Fig. 2). Stomatal density was quantified on both adaxial and abaxial epidermal surfaces by imaging freshly detached leaves using the Keyence VHX-7000 digital microscope (Keyence Corporation, Osaka, Japan), a high-resolution imaging system equipped with variable magnification and depth composition capabilities. For each treatment, 12 cotyledons and 12 true leaves were analysed. Images were acquired at 500×

magnification from three randomly selected areas on each surface and leaf type, and stomata were counted to calculate stomatal density (number of stomata per mm^2).

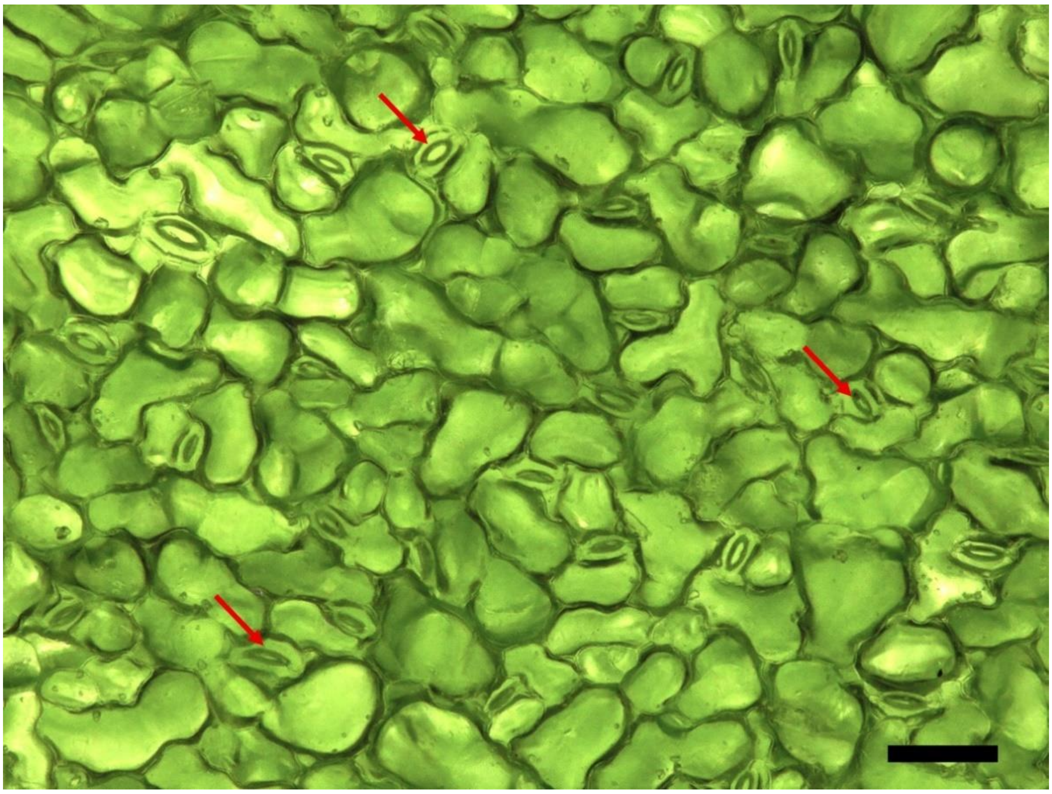

Figure 2. Microscopic view of stomata on freshly detached leaf epidermis, acquired using the Keyence VHX-7000 digital microscope. Images were used to determine stomatal density. Red arrows indicate stomata. Black bar = 50 µm

3.3.5. Statistical analysis

All data were analyzed to assess the effects of substrate type on early development of *Borago officinalis*. Statistical analyses were conducted using IBM SPSS Statistics (version 29.0.2.0, IBM Corp., Armonk, NY, USA) and R version 3.5.2. (R Core Team 2024).

To assess the effects of substrate type on germination and seedling development of *B. officinalis* (i.e., germination, cotyledon emergence/opening, leaves emergence, and seedling mortality), we applied Survival Analysis, a time-to-event statistical framework well-suited to binary outcomes over time and to manage censored data (i.e., observations where the event did not occur during the study period; Romano and Stevanato 2020). We performed a Kaplan–Meier (KM) survival curves (Keplan and Meier 1958) as non-parametric estimator of the survival function to provide a stepwise approximation of the probability of not experiencing the event at a given time point (Goel et al. 2010; Roesler et al. 2023). We fitted Accelerated Failure Time (AFT) models as fully parametric regressions to quantify how substrate type affects the time to event occurrence (Onofri et al. 2010; Romano and Stevanato 2020; Silvestri et al. 2020). In our case, SO was used as the reference treatment. Additionally, we performed pairwise comparisons using AFT models, allowing direct contrasts between all treatment pairs. This approach improved model fit and provided more detailed insights into substrate-specific effects across stages. Model diagnostics and assumption were checked prior to model selection. Although Cox proportional hazards models were initially considered, the proportionality assumption was violated, favoring the use of fully parametric AFT models.

Growth, physiological, and anatomical parameters were analyzed using analysis of variance (ANOVA). Prior to analysis of variance, the assumptions of normality and homogeneity of variances were tested using the Shapiro–Wilk and Levene's tests, respectively. For variables that met parametric assumptions, one-way analysis of variance (ANOVA) was applied, followed by Tukey's HSD post hoc test for pairwise comparisons among substrate treatments. In cases where assumptions of normality or homoscedasticity were violated, a robust ANOVA (Welch's ANOVA) was performed, followed by Games–Howell post-hoc test. Statistical significance was set at $P < 0.05$ for all tests.

3.4. Results

3.4.1. Growth

Germination of *B. officinalis* started 4 days after sowing (DAS) in all treatments. Kaplan–Meier (KM) survival curves (Keplan and Meier 1958) visually highlight differences among substrate types for all events (germination, cotyledon emergence, leaves emergence, and mortality). The SO treatment promoted a faster progression of developmental stages and slower mortality, as indicated by the steeper decline of the survival curves over time (Fig 3). In contrast, AS induced a sharp decrease in survival, indicating rapid and early mortality (Fig. 3). RS produced an intermediate mortality trend, with faster mortality than SO but slower than AS, whereas SS displayed a pattern closer to the control, with no significant difference compared to SO (Fig. 3), in accordance with the AFT model results.

Pairwise comparisons between treatments based on the Accelerated Failure Time (AFT) model with a Weibull distribution revealed that the SO substrate significantly accelerated germination compared to all other substrates. Specifically, SO reduced the median germination time by 61% compared to AS ($p < 0.0001$; Table S1), 41% compared to RS ($p = 0.0108$), and 56% compared to SS ($p = 0.0001$; Table S1).

No significant differences were found between AS and SS and between RS and SS ($p > 0.1$ Table S1). In all these comparisons, the relative time ratio was < 1, indicating a faster germination process in SO compared to the other treatments (Table S1).

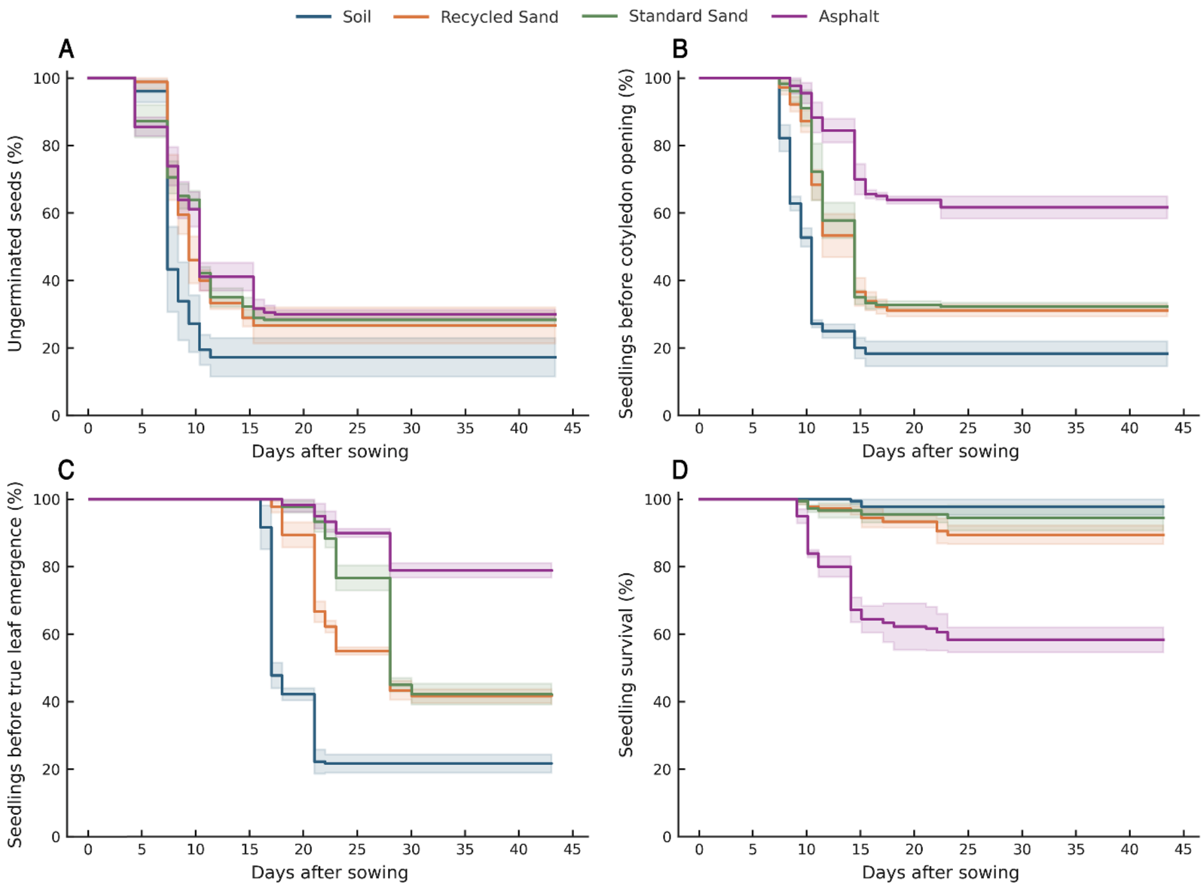

Figure 3. Kaplan–Meier curves comparing substrate effect on four seedling developmental stages survival time. (A) Ungerminated seeds (%): the survival curve tracks the proportion remaining ungerminated (event correspond to germination); (B) Seedlings before cotyledon opening (%): the curve tracks the proportion prior to opening (event correspond to cotyledons emergence); (C) Seedlings before true leaf emergence (%): the curve tracks the proportion prior to true leaf emergence (event correspond to leaves emergence); (D) Seedling survival (%): the curve tracks the proportion alive (event correspond to mortality). Lines show the mean across four replicates; shaded ribbons are 95% confidence intervals. Measurements were taken from sowing (Day 0) up to Day 43. Substrate treatments include Soil (blue line), Recycled Sand (orange line), Standard Sand (green line), and Asphalt (purple line). Time is expressed as days after sowing (DAS); all series start at 100% at $t = 0$. These visual patterns align with Weibull AFT model results.

The AFT model with a Weibull distribution also revealed significant differences in cotyledon emergence time across substrate treatments. Compared to SO, all substrates significantly delayed the emergence of cotyledons ($p < 0.001$; Table S1). AS showed the most pronounced delay, with a relative time (ratio = 0.159; Table S1), while RS (ratio =

0.526; Table S1) and SS (ratio = 0.498; Table S1) also significantly delayed emergence. No significant difference was found between RS and SS ($p = 0.719$).

Additionally, the model revealed a highly significant effect of substrate type on the timing of true leaf emergence. Estimated median emergence times differed markedly among treatments, as confirmed by relative time ratios of the pairwise comparison, with AS (ratio = 0.329; $p < 0.0001$; Table S1), RS (ratio = 0.672; $p < 0.0001$; Table S1) and SS (ratio = 0.641; $p < 0.0001$; Table S1) showed significant delays relative to SO. However, no significant difference was detected between RS and SS ($p = 0.492$; Table S1).

Furthermore, a strong effect of substrate type on the timing of seedling mortality were clearly revealed. Seedlings grown on AS experienced early mortality (ratio = 63.9), as a relative time greater than 1 indicates markedly faster progression toward death compared with SO ($p < 0.0001$; Table S1). Mortality was also significantly faster in RS (ratio = 7.8, $p = 0.0046$; Table S1) compared to SO, while SS showed an intermediate response (time ratio = 3.3) that did not differ significantly from the control baseline ($p = 0.12$; Table S1). All significant statistics remained significant after corrections (i.e., FDR and Bonferroni), confirming the robustness of the results.

3.4.2. Biomass accumulation

Substrate type had a strong influence on biomass accumulation over time (Fig. 4). Fresh and dry weights of both roots (Fig. 4A, 4B) and shoots (Fig. 4C, 4D) were consistently higher in SO plants compared to RS and SS, and markedly greater than AS at both time points. Increases in root and shoot biomass between 20 DAS and 43 DAS were most pronounced in plants grown on SO, followed by those on sand-based substrates. Minimal biomass gains were observed in AS plants, and particularly for roots (Fig. 4A, 4B).

The root-to-shoot ratio also varied significantly among treatments (Fig. 4E). Plants grown on RS showed the highest roots-to-shoots values, followed by those grown on SO, whereas plants grown on SS and AS exhibited the lowest values (Fig. 4E).

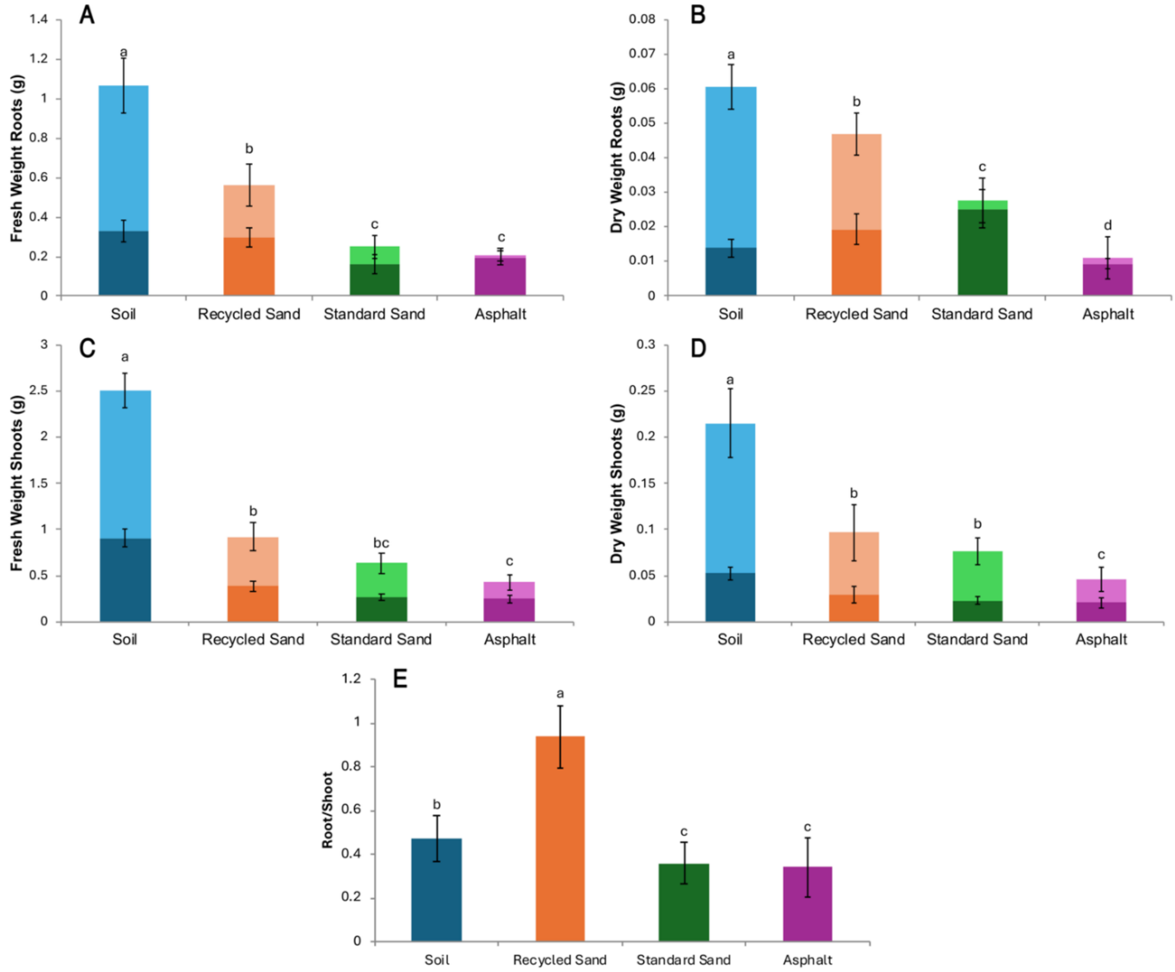

Figure 4. Root and shoot biomass accumulation of *Borago officinalis* seedlings grown on four different substrates. Panels show fresh weight of roots (A), dry weight of roots (B), fresh weight of shoots (C), dry weight of shoots (D), and root/shoot ratio (E). Bars with two colours represent measurements at 20 DAS (darker colour) and 43 DAS (lighter colour). Substrate treatments include Soil (blue), Recycled Sand (orange), Standard Sand (green), and Asphalt (purple). Letters indicate statistically significant differences at $P < 0.05$ based on post-hoc comparisons.

3.4.3. Plant physiology

Chlorophyll *a* fluorescence and gas exchange parameters exhibited substrate-dependent variation at both the cotyledon and true leaf stages. Stomatal conductance (g_s) and transpiration rate (E) in cotyledons differed significantly among treatments, with plants grown on RS, SS and AS showing higher values compared to those grown on SO (Table 1). In contrast, no differences among SO, RS, SS, and AS were observed for F_v/F_m, QY, NPQ, or leaf surface temperature (Table 1).

At the leaf stage, more pronounced differences were observed. QY was significantly reduced in Asphalt-grown plants compared to SO, while intermediate values were observed in both sand treatments (Table 1). NPQ was lowest in SO, indicating a reduced level of non-photochemical quenching compared to the other treatments. Differently to what was observed in the cotyledons, stomatal conductance and transpiration rate were both significantly higher in soil-grown plants compared to all other treatments, and surface temperature was correspondingly lower in these plants (Table 1).

Table 1. Photosynthetic and gas-exchange parameters (mean ± standard deviation; n = 12) of plants grown on different substrates. Different letters indicate statistically significant differences among treatments ($P < 0.05$).

Stage	Parameter	Soil	Recycled Sand	Standard Sand	Asphalt
Cotyledons	F_v/F_m	0.76 ± 0.01	0.76 ± 0.01	0.75 ± 0.02	0.75 ± 0.02
	QY	0.57 ± 0.10	0.61 ± 0.06	0.61 ± 0.07	0.60 ± 0.07
	NPQ	0.58 ± 0.39	0.56 ± 0.28	0.44 ± 0.20	0.38 ± 0.17
	g_s	0.11 ± 0.05 b	0.18 ± 0.05 a	0.19 ± 0.10 a	0.18 ± 0.06 a
	E	1.26 ± 0.56 b	2.03 ± 0.52 a	2.10 ± 0.58 a	2.19 ± 0.59 a
	Surface Temperature	20.7 ± 0.89	20.5 ± 0.43	20.6 ± 0.63	21.1 ± 0.45
Leaves	F_v/F_m	0.72 ± 0.07	0.72 ± 0.04	0.73 ± 0.06	0.76 ± 0.05
	QY	0.52 ± 0.04 a	0.50 ± 0.05 ab	0.51 ± 0.04 ab	0.47 ± 0.06 b
	NPQ	1.61 ± 1.05 b	2.78 ± 0.65 a	2.65 ± 1.26 a	2.89 ± 1.07 a
	g_s	0.13 ± 0.05 a	0.07 ± 0.02 b	0.08 ± 0.03 b	0.08 ± 0.04 b
	E	1.95 ± 0.47 a	1.30 ± 0.36 b	1.39 ± 0.49 b	1.28 ± 0.35 b
	Surface Temperature	24.9 ± 1.20 b	27.9 ± 1.70 a	27.3 ± 1.65 a	27.4 ± 1.44 a

3.4.4. Morpho-anatomical traits

Substrate type significantly influenced morphological and anatomical traits of the seedlings. Cotyledon area was highest in SO (Table 2), significantly greater than all other treatments. RS and SS showed intermediate values with no statistical difference between them, while AS had the smallest cotyledon area (Table 2). A similar trend was observed for true leaves. Soil-grown plants exhibited the largest leaf area, with values substantially higher than those measured in plants grown on other treatments (Table 2). Specifically, leaf area in soil-grown plants was approximately 2.6 times greater than in RS, 3.8 times greater than in SS, and 5.7 times greater than in AS (Table 2).

Leaf Mass per Area (LMA) showed some variation among the four tested substrates (Table 2). The highest values were recorded in plants grown on SS, while the lowest were observed in those grown on SO. RS and AS resulted in intermediate values, not significantly different from either SS or SO (Table 2).

Regarding stomatal density, plants grown on AS, RS, and SS exhibited higher values than those grown on SO across both abaxial and adaxial surfaces of cotyledons and true leaves, except for the adaxial surface of true leaves, where no significant differences were detected (Table 2).

Table 2. Morphological and anatomical parameters (mean ± standard deviation; n = 12) of plants grown on different substrates. Different letters indicate statistically significant differences among treatments ($P < 0.05$).

Parameter	Soil	Recycled Sand	Standard Sand	Asphalt
Cotyledon surface area (cm^2)	12.88 ± 2.27 a	6.59 ± 1.12 b	3.81 ± 0.52 c	4.19 ± 0.74 c
Leaf area (cm^2)	21.40 ± 3.03 a	8.37 ± 1.47 b	5.58 ± 1.88 bc	3.75 ± 1.00 c
Cotyledon abaxial stomatal density (n/mm^2)	71.0 ± 12.6 b	102.2 ± 14.4 a	95.3 ± 10.4 a	110.1 ± 16.5 a
Cotyledon adaxial stomatal density (n/mm^2)	52.7 ± 6.0 b	71.5 ± 6.2 a	67.0 ± 6.3 a	77.9 ± 5.2 a
Leaf abaxial stomatal density (n/mm^2)	133.3 ± 24.9 b	174.3 ± 16.3 a	183.9 ± 23.4 a	188.5 ± 20.3 a
Leaf adaxial stomatal density (n/mm^2)	89.4 ± 23.8	94.5 ± 15.8	104.1 ± 16.4	107.0 ± 22.6
Leaf mass per area (g/m^2)	103.8 ± 14.2 b	115.1 ± 18.4 ab	137.9 ± 17.7 a	124.0 ± 18.6 ab

3.5. Discussion

UGS are often created through reclamation of brownfields and/or removing sealing paving from abandoned areas (Cortinovis et al. 2022; Rompato et al. 2025). Using topsoil imported from rural or natural areas for urban soil construction is often economically and environmentally costly and conflicts with circular economy principles (Rokia et al. 2014; Deeb et al. 2020; González-Méndez and Chávez-García 2020). Concurrently, urban construction sites generate substantial amounts of construction-derived waste from demolitions and excavations, whose relocation imposes significant costs for public administrations (Guilland et al. 2018). Moreover, urbanization degrades soil quality, reducing its ability to deliver essential ecosystem services, such as urban agriculture, water infiltration, carbon sequestration, and greening of urban infrastructure (Guilland et al. 2018; Mikajlo et al. 2025).

Recycling urban waste to construct soils represents a promising approach to simultaneously address landfill disposal challenges and limit the overexploitation of natural topsoil (Séré et al. 2008; Deeb et al. 2020; Fabbri et al. 2021). Locally available recycled materials such as

CDWs are increasingly considered for these applications (Abbruzzini et al. 2022). However, research on how these materials affect plant growth and physiology remains limited.

Recent experimental studies demonstrate fast seedling growth and high plasticity of *B. officinalis* under different conditions (Abd El-Rahman et al. 2023; Rostampour et al. 2023; Falcinelli et al. 2024), confirming its adaptability for varied conditions and its suitable nature for urban environment. This plasticity in anatomical responses was useful to provide valuable insight into how urban substrates can affect early seedling development.

Indeed, our results demonstrated that substrate types significantly influence the early development of *B. officinalis* seedlings, corroborating previous findings that urban pollutants, especially those associated with AS, can restrict plant growth by negatively affecting leaf size, chlorophyll content, stomatal dimensions, and density (Ajuru and Upadhi 2016). Seedlings grown on AS showed the latest germination and emergence rates, the fastest mortality, and limited biomass accumulation, supporting the hypothesis that compacted, impervious, and potentially contaminated substrates impose abiotic stresses, such as limited water availability that hinder seedling establishment and resource acquisition.

Specifically, the Survival analysis showed that germination of *B. officinalis* occurs significantly faster in SO than in any of the alternative substrates, while similar germination dynamics were found between AS and SS, as well as between RS and SS. Our findings align with previous studies, suggesting that alternative substrates may pose physical or chemical constraints on germination by displaying altered porosity, compaction, or nutrient dynamics (Tzoulas et al. 2007; Dayton et al. 2010; Mullaney et al. 2015; Fabbri et al. 2021; Abbruzzini et al. 2022; Mikajlo et al. 2025).

Similarly, cotyledons emerged up to six times more slowly in AS than in SO, while sand-based substrates roughly doubled the time compared to SO. Moreover, AS, RS and SS showed a significant delay in true leaves emergence relative to SO, showing that early developmental constraints persist beyond germination. Asphalt-based substrates also significantly delayed cotyledon and leaf emergence compared to both sand-based treatments, whereas no significant difference was detected between the two sand substrates, suggesting similar performance between them. This is consistent with broader evidence that urban soils and constructed substrates often suffer from reduced organic matter, poorer microbial activity, and impaired nutrient cycling (Lehmann and Stahr 2007; Pouyat et al. 2010; Morel et al. 2015; de Barros Ruas et al. 2022).

The mortality patterns underline the detrimental effect of AS, where a rapid decline in survival was observed from the early stages, suggesting that this substrate creates unsuitable conditions to seedling persistence probably due to high surface temperature, low porosity and limited water retention capability (Ajuru and Upadhi 2016; Yilmaz et al. 2018), which can lead to severe water stress and root damage (Mullaney et al. 2015; Itodo et al. 2018). However, since substrate properties were not directly assessed in the present work, future investigations could benefit from including physical and chemical analyses of substrates to better elucidate how factors such as surface temperature, porosity, and water retention can influence *B. officinalis* seedling establishment in urban environments. Accordingly, SO-grown plants exhibited significantly larger cotyledons and true leaves, the highest biomass accumulation, and the most favourable physiological traits, whereas plants on AS and SS developed smaller leaves and cotyledons, probably as a drought avoidance strategy to minimize transpiration surface under unsuitable substrate conditions. The higher leaf mass per area (LMA) that we found in plants on AS and SS also supports this interpretation, pointing to an investment in structurally dense tissues, a typical adaptation in species occurring under conditions of reduced water availability (De La Riva et al. 2016). This aspect further suggests that the alternative substrates tested possess low structural quality, however, additional physical and chemical analysis of these substrates are required to confirm this hypothesis.

Interestingly, RS and SS showed intermediate responses between AS and SO in survival patterns and leaf traits, suggesting that these substrates provide more favourable conditions than asphalt-based treatments. As already shown in previous studies, our results confirm that under suboptimal substrate conditions, particularly low nutrient availability, plants increase their root-to-shoot ratio as an adaptive strategy to improve nutrient uptake (Delerue et al. 2022). Although still suboptimal compared to SO, both sand treatments supported relatively high germination and emergence rates, and moderate biomass accumulation, suggesting their potential as viable components of constructed technosols for UGS. Therefore, being composed exclusively by mineral fraction, the absence of organic matter might limit water retention and nutrient availability (Dayton et al. 2010; De Koff et al. 2010), ultimately resulting in survival times still significantly shorter than in SO.

However, the relatively low biomass in SS and reduced root development in both sands highlight the need for further improvement, such as the addition of organic amendments, to fully support plant growth and development (Brusselaers and Van Der Linden 2020; Rompato et al. 2025). Indeed, previous studies have suggested that the partial incorporation of recycled materials into anthroposols can promote more sustainable urban soil and waste management (Guilland et al. 2018; Deeb et al. 2019; Prado et al. 2020; Jia et al. 2024; Rompato et al. 2025), and have also recommended improving their quality through the addition of organic amendments (De Koff et al. 2010; Coull et al. 2021; Abbruzzini et al. 2022; Mikajlo et al. 2025).

Morpho-anatomical traits observed in our experiment can be considered as early indicators of plant stress and functional adjustment. The consistent reduction in leaf area and cotyledon size in AS and SS plants likely represent a drought-avoidance strategy, whereby seedlings limit transpiring surface area to reduce water loss under substrate-induced stress (Driesen et al. 2023). Stomatal traits were also modulated by substrate-induced stress. Although stomatal density is often genetically fixed, it can exhibit plasticity in response to environmental cues such as light, carbon dioxide, and water availability (Hetherington and Woodward 2003; Casson and Gray 2008; Xu et al. 2016). In our case, SO-grown plants exhibited the lowest stomatal densities, consistent with efficient gas exchange regulation. In contrast, plants grown on CDWs substrates showed significantly higher stomatal densities. This trend might reflect a compensatory response to substrate-related limitations in water and nutrients, as previously observed under abiotic stresses such as drought and salinity (Koevoets et al. 2016). In addition, increased stomatal density under abiotic stress has often been associated with restricted leaf expansion and smaller epidermal cells, leading to a higher number of stomata per unit area under water deficit (Pirasteh-Anosheh et al. 2016; Caine et al. 2023). Accordingly, the increased stomatal density and the reduced leaf area found in plants grown on suboptimal substrates might be a plastic adjustment aimed at maintaining gas exchange under constrained resource availability. Indeed, our plants on SO displayed higher stomatal conductance and transpiration rates, along with lower leaf temperatures, indicating efficient evaporative cooling and photosynthetic performance. Conversely, AS-grown plants exhibited reduced gas exchange capacity and lower quantum yield (QY), in line with previous evidence that the compact and harsh nature of asphalt can impact photosynthetic efficiency and growth (Ajuru and Upadhi 2016; Itodo et al. 2018). Nonetheless, future studies could include comprehensive assessments of substrate characteristics to better clarify their roles in shaping plant trait responses and to strengthen the reliability of ecological evaluations.

Overall, these results indicate that substrate composition substantially affects early plant physiological processes. AS can significantly slow down germination, cotyledons, and true leaf development compared to the SO, with potential implications for seedling establishment in urban or disturbed environments. SO also ensures prolonged survival, emphasizing the crucial role of the substrates properties in supporting the regeneration and persistence of *B. officinalis* under stressful conditions. Moreover, the strong response of *B. officinalis* to substrate type underlines its potential use as a sensitive bioindicator for substrate degradation and restoration efficiency in disturbed environments, such as the urban environments.

From an applied perspective, our findings underscore the importance of substrate-specific selection in urban soil reconstruction. AS showed limited suitability for plant development, whereas RS performed better than SS in biomass allocation, and should be favored since it also comes from recycled material. Besides, the selection and mixing of CDW-based substrates, ideally supplemented with compost or organic amendments, may result efficient in supporting plant establishment and development, but this aspect requires more investigation. In this context, monitoring early plant responses can offer a rapid and cost-effective means to evaluate substrate performance in real or simulated urban environments. This study supports more sustainable, informed choices for UGS. Moreover, it highlights the often-overlooked role of urban soils in sustaining ecosystem services, including habitat provision for biodiversity, and urban agriculture, essential components of nature-based urban ecosystems (Guilland et al. 2018). Given the large volumes of inert waste generated by ongoing construction, the reuse of recycled materials as functional components of green infrastructure aligns with circular economy principles. When appropriately managed, these materials can reduce landfill demand and limit the overexploitation of natural soils while simultaneously enhancing urban ecological function. By facilitating plant growth, improving soil health, supporting biodiversity, and contributing to climate regulation, such approaches can also enhance the aesthetic and recreational value of urban environments. Incorporating substrate-specific design and monitoring into UGS planning not only improves the ecological performance of vegetation but also supports the achievement of several UN Sustainable Development Goals (SDGs): Good Health and Well-being (SDG 3), Industry, Innovation and Infrastructure (SDG 9), Sustainable Cities and Communities (SDG 11), Responsible Consumption and Production (SDG 12), Climate Action (SDG 13), and Life on Land (SDG 15). Ultimately, this research contributes to a growing body of evidence that urban substrate design is a critical, yet underutilized, tool for building sustainable and resilient cities.

3.6. References

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Chapter 4

Conservation in European cities: Assessing the Role of Urban Habitat Heterogeneity in Supporting Threatened Plant Species

4.1. Abstract

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4.2. Introduction

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4.3. Materials and methods

4.3.1. Data collection

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4.4. Results

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4.5. Discussion

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4.6. References

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[redacted] https://doi.org/10.1002/fee.1480

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[redacted] https://doi.org/10.1016/j.jag.2017.12.009

[redacted] https://doi.org/10.1111/jbi.13591

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Chapter 5

General comments and considerations

This Ph.D. project was funded by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union - NextGenerationEU. In particular, the project was carried out within the activities of spoke 5 (urban environment and health) of the National Biodiversity Future Centre (NBFC).

The work progressed in different phases and consisted of research activities on different research topics, scientific dissemination, international collaborations and conferences. This Ph.D. experience gave me the opportunity to develop knowledge, methodologies and new interest concerning research in plants science.

The research activities carried out during the Ph.D fully comply with the missions outlined in Spoke 5 of the NBFC, improving knowledge to enhance the environmental and biodiversity resources of the city, promoting biological complexity and addressing the gaps and opportunities of the urban ecosystems.

Moreover, results obtained contribute to supporting the achievement of several UN Sustainable Development Goals (SDGs) by advancing knowledge and practical solutions, particularly for Good Health and Well-being (SDG 3), Sustainable Cities and Communities (SDG 11), Climate Action (SDG 13) and Life on Land (SDG 15).

During this Ph.D project, I investigated urban plant biodiversity by different approaches and prospectives. Research activities were conducted on plant biodiversity and ecology in urban environments. Particularly, I analyzed the role of plants in cities, the ecological functions of green spaces, the effects of urbanization on plant growth and development, and possible conservation value of the cities.

An in-depth literature review regarding plant biodiversity in urban environment was carried out and the critical points of the research in progress were evaluated. Whitin this review the dual and interconnected role of plants has emerged: plants are organisms both strongly threatened by urban stress and concurrently indispensable for revealing and relieving urban negative effects. Consequently, urban plants can serve as bioindicators and essential mitigators of environmental changes. In the review, I also underlined how the relationship between human and nature can be considered as symbiotic especially within the cities, where the persistence of nature depends largely on human actions and management, as well as the human physical, mental, and social well-being is closely tied to the presence of urban vegetation and biodiversity.

One of the research activities focused on the species of the Royal Park of Portici, an historic urban forest that, being planted 300 years ago. It represented an important case study for assessing contribution and the long-term ecological effects of planting new urban forest to urban biodiversity. By analysing plant traits in a 300-year-old urban forest, insights were provided for plant conservation in compact cities. Results indicated a strong ecological segregation driven by urbanization, which acts as a selective force promoting species adapted to disturbance, while filtering out those that require more stable habitat continuity. It was concluded that urban forests have critical roles as refugia for species with specific ecological needs, and as a source of species for urban biodiversity.

Specific experiments carried out during the Ph.D. period considered the impact of construction-derived substrates on seed germination and early development of *Borago officinalis*. The main approaches used to develop the research activities were focused on morphological, physiological and anatomical responses of plants to the different substrates' treatments. It was underscored the importance of substrate-specific selection in urban soil reconstruction and suggested that selection and mixing of CDW-based substrates, ideally supplemented with compost or organic amendments, may result efficient in supporting plant establishment and development.

In addition to the literature review, species sampling and experiments, some activities were dedicated to evaluate conservation opportunities within European cities. These activities were performed assessing the role of different urban habitat types in supporting threatened species. The analysis of urban floras from different environments in more than 50 European cities allowed to highlight the importance of habitat availability and management for the conservation of threatened species.

In the context of urban plant biodiversity, an overall conclusion was that the urban environment represents a critical and expanding frontier for the study of plant biodiversity. Cities function as open-air, low-cost laboratories where the effects of climate change are replicated and often intensified, offering unique opportunities to observe ecological and evolutionary dynamics as they unfold.

As urban areas are characterized by multiple environmental stressors and conditions, understanding how plant species and communities respond to fragmentation, pollution, microclimatic variability, soil alteration, and human management remains essential for predicting future trajectories of urban biodiversity and for designing cities capable of withstanding ongoing biodiversity loss. Overall, there is no doubt that plants are fundamental components of the cities. With most of the global population now living in urban areas, plant diversity provides indispensable ecosystem services that support human well-being, holding ecological, genetic, economic, scientific, educational, cultural, recreational, and aesthetic value as well as an intrinsic worth coming from long evolutionary processes. Therefore, conserving and promoting biodiversity within cities is a matter of environmental concern, as well as an ethical commitment, which human beings cannot ignore.

Downstream from the entire study process, I argue that the most recent knowledge in urban ecology must be translated into planning, policy, and practice. Urban green spaces must be increased, varied, connected, and appropriately managed, according to expert advice. They should not be considered only as ornamental places, or alternative refuges from urban life, but as integral, essential components and active infrastructure of the city. Plants, a fundamental component of biodiversity and bearers of ecosystem services, must be placed at the center of urban planning. Adapting cities (and the mindset of their inhabitants) to nature needs, rather than attempting to adapt nature to cities, will be fundamental to achieving sustainable urban development, ensuring prosperity for biodiversity and well-being for future generations.

Among all activities, results obtained during these three years of research were presented at several international conferences (EcoFlor 24, X IPSC, XI IPSC). In addition, the topics covered during the Ph.D. program were deepened thanks to the participation in high-level courses such as the "Training school in Biology and Pollination Ecology (Module II)", placed at the Departments of Life Sciences and Botanical Garden, University of Coimbra (Portugal) and "Corso Base di R 2023 Bis", promoted by Successione Ecologica APS and placed at the CNR in Sesto Fiorentino (Italy).

The opportunities provided by this Ph.D. program allowed me to expand networks, knowledge and familiarity of scientific community by collaborating with other institutes and researchers.

Appendix

Publications

- Rummo, R., Fantinato, E., & Aronne, G. (2025). Plant traits in a 300-year-old urban forest: insights for plant conservation in compact cities. *Journal of Urban Ecology*, *11*(1), juaf013. https://doi.org/10.1093/jue/juaf013
- Rummo, R., Izzo, L. G., & Aronne, G. (2026). Impact of construction-derived substrates on seed germination and early development of Borago officinalis: insight for sustainable management of urban green spaces. *Urban Ecosystems*, *29*(1), 2. https://doi.org/10.1007/s11252-025-01862-9

Courses

- English language, Department of Agricultural Sciences, University of Naples Federico II
- Ecologia e Biometria (Corso Integrato), Dipartimento di Scienze E Tecnologie Ambientali Biologiche E Farmaceutiche (Distabif), Università degli studi della Campania Luigi Vanvitelli
- Biometria, Dipartimento di Scienze E Tecnologie Ambientali Biologiche E Farmaceutiche (Distabif), Università degli studi della Campania Luigi Vanvitelli
- How To Write a Scientific Paper, Department of Agricultural Sciences, University of Naples Federico II
- Laboratorio di Ecologia Quantitative (Modulo Corso Integrato Analisi dei Dati Ecologici, Department of Agricultural Sciences, University of Naples Federico II
- Training school in Biology and Pollination Ecology (Module II). Promoted by the COST Action ConservePlants CA18201- An integrated approach to conservation of threatened plants for the 21st Century. Departments of Life Sciences and Botanical Garden, University of Coimbra, Coimbra,Portugal
- Corso Base di R 2023 Bis, Dott. Jacopo Cerri, Successione Ecologica APS, CNR Sesto Fiorentino

Seminars

- Vertical farming: Beyond the hype. Leo Marcelis, Wageningen University, NL. 20 February 2024
- Why agriculture is not a viable economic activity in present-day society: Implications and reflections. Mario Giampietro, Institució Catalana de Recerca i Estudis Avançats Barcelona, ES. 10 June 2024

- Role and function of the FAO Global Information and Early Warning System (GIEWS) on food and agriculture. Mario Zappacosta, FAO, Rome, IT. 08 July 2024
- Zero Tillage: the good, the bad and the ugly. Sacha Mooney, University of Nottingham, UK. 16 October 2024

Oral presentation

- Rosario Rummo, Edy Fantinato, Giovanna Aronne, "*Identifying plant traits for sustainable green infrastructure in compact urban environments: a case study of Portici, Italy*". 118° Congresso della Società Botanica Italiana (X International Plant Science Conference (IPSC), Aula Magna dell'Università di Teramo Campus "Aurelio Saliceti", Teramo

Poster

- Rummo R., Fantinato E., Aronne G.: *Plant reproductive, vegetative, and dispersal traits to investigate urban biodiversity and plant ecological function*, Ecoflor 24: 21st Annual Meeting of the Spanish Group for Floral Ecology, Departments of Life Sciences and Botanical Garden, University of Coimbra, Coimbra, Portugal; 01-02-2024
- Rummo R., Izzo L.G., Aronne G. "Effects of urban soil components on germination and early development of Borago officinalis: implications for urban green space management". 120° Congresso della Società Botanica Italiana (XI International Plant Science Conference – IPSC), GoBot2025 Overcoming Barriers in Plant Science and Beyond Gorizia, September 3-6, 2025.

Teaching support

- Biologia ed Ecologia della Riproduzione delle Piante (Prof. G. Aronne), support for laboratory and field practical classes
- Botanica Generale e Sistematica (Prof. G. Aronne), support for laboratory practical classes and lectures

Given seminary

- Rummo R., "Growing in space: the role of plants (and botanists) in space mission", Department of Biodiversity at UP FAMNIT, University of Primorska, faculty of mathematics, natural sciences and information technologies, Department of biodiversity, Kampus Livade, Izola, Slovenia, 26 November 2024

Side Study within the spoke 5 of the National Biodiversity Future Center (NBFC)

- Study on intraspecific differences in seed production and germinability between urban and natural environments. Samling of *Trifolium pratense* and *Raphanus raphanistrum* in Urban and Natural environments of Naples and Milan. In vitro germination test to compare the effect of three different temperatures on seed germination.

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