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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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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.

Keywords Borago officinalis · Circular economy · Seedling development · Survival analysis · Urban green spaces · Urban wastes

Introduction

Urbanization is the fastest-growing form of land use worldwide (Lin and Fuller 2013) and represent a significant anthropogenic disturbance for plants. Globally, urban inhabits 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

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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 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 (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).

Materials and methods

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).

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

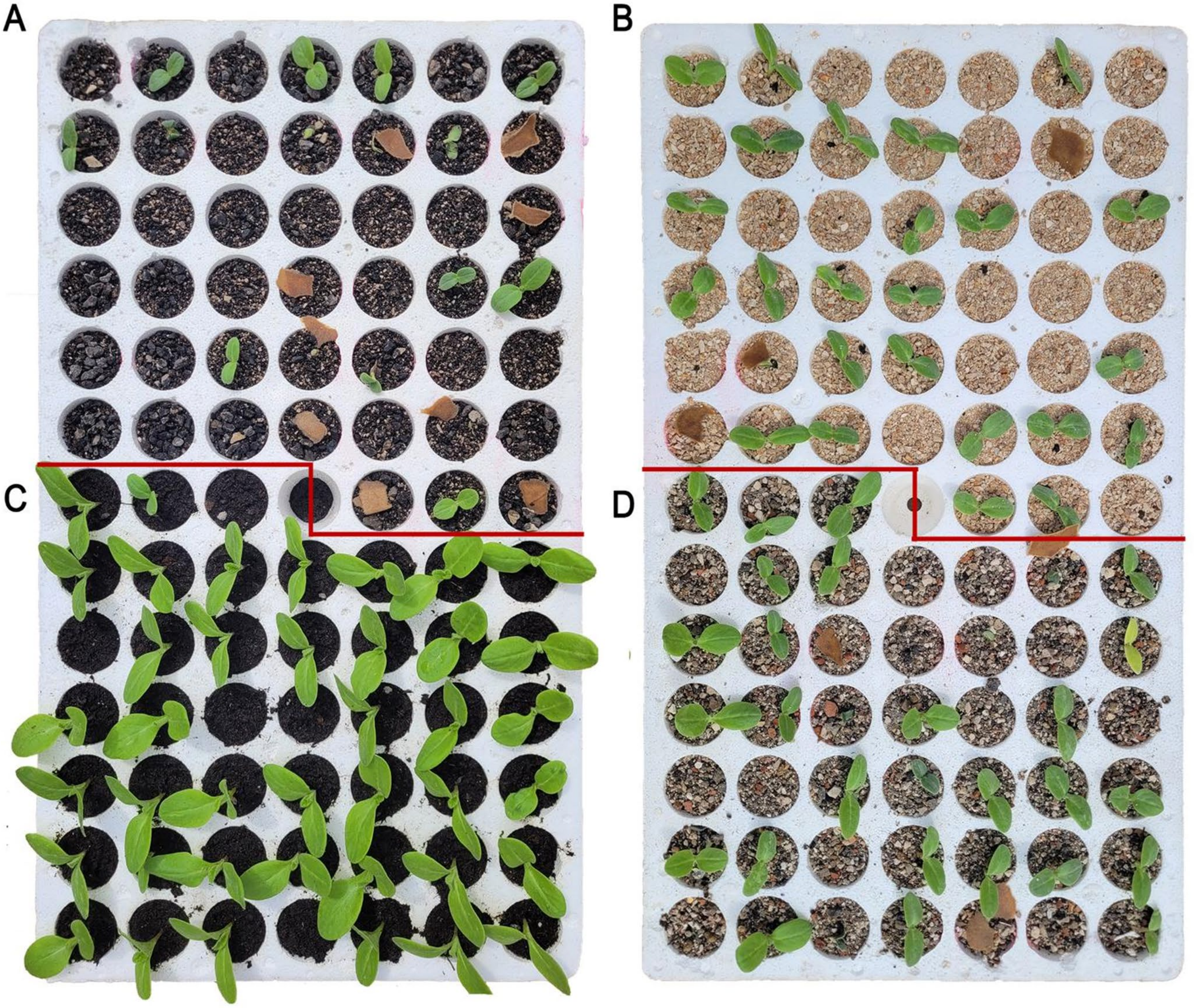

Fig. 1 Experimental setup and polystyrene tray used in the study as example of substrate allocation: (**A**) asphalt; (**B**) standard sand; (**C**) potting soil; (**D**) recycled sand

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 h 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.

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).

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 mm²).

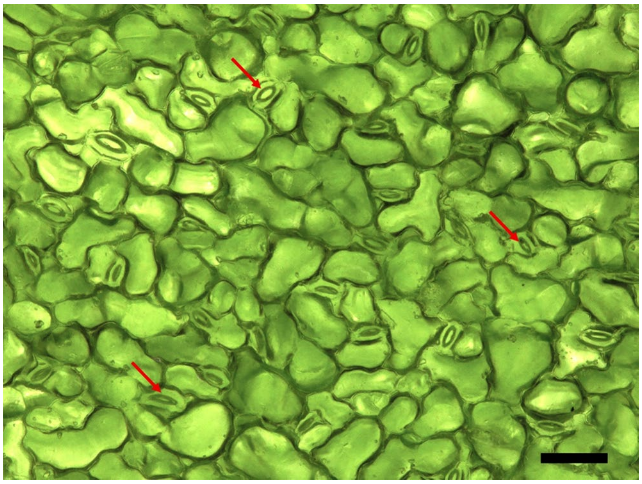

Fig. 2 Microscopic view of stomata on freshly detached leaf epidermis, acquired using the Keyence VHX-7000 digital microscope. Images were used to determine stomatal density. Red arrows indicate stomata. Black bar = 50 µm

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 provides 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.

Results

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).

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;

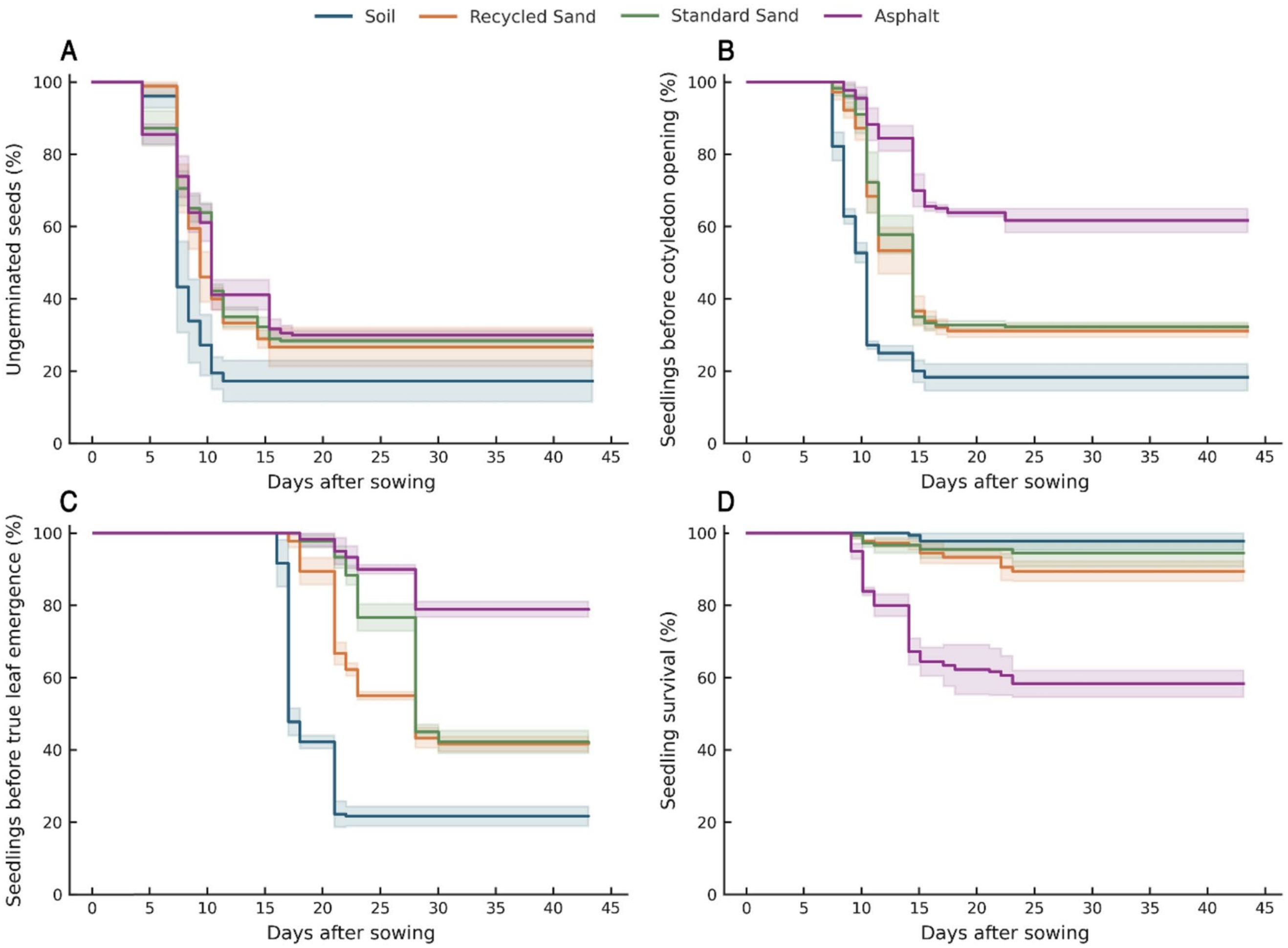

Fig. 3 Kaplan–Meier curves comparing substrate effect on four seedling developmental stages survival time. (**A**) Ungerminated seeds (%): the survival curve tracks the proportion remaining ungerminated (event correspond to germination); (**B**) Seedlings before cotyledon opening (%): the curve tracks 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

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.

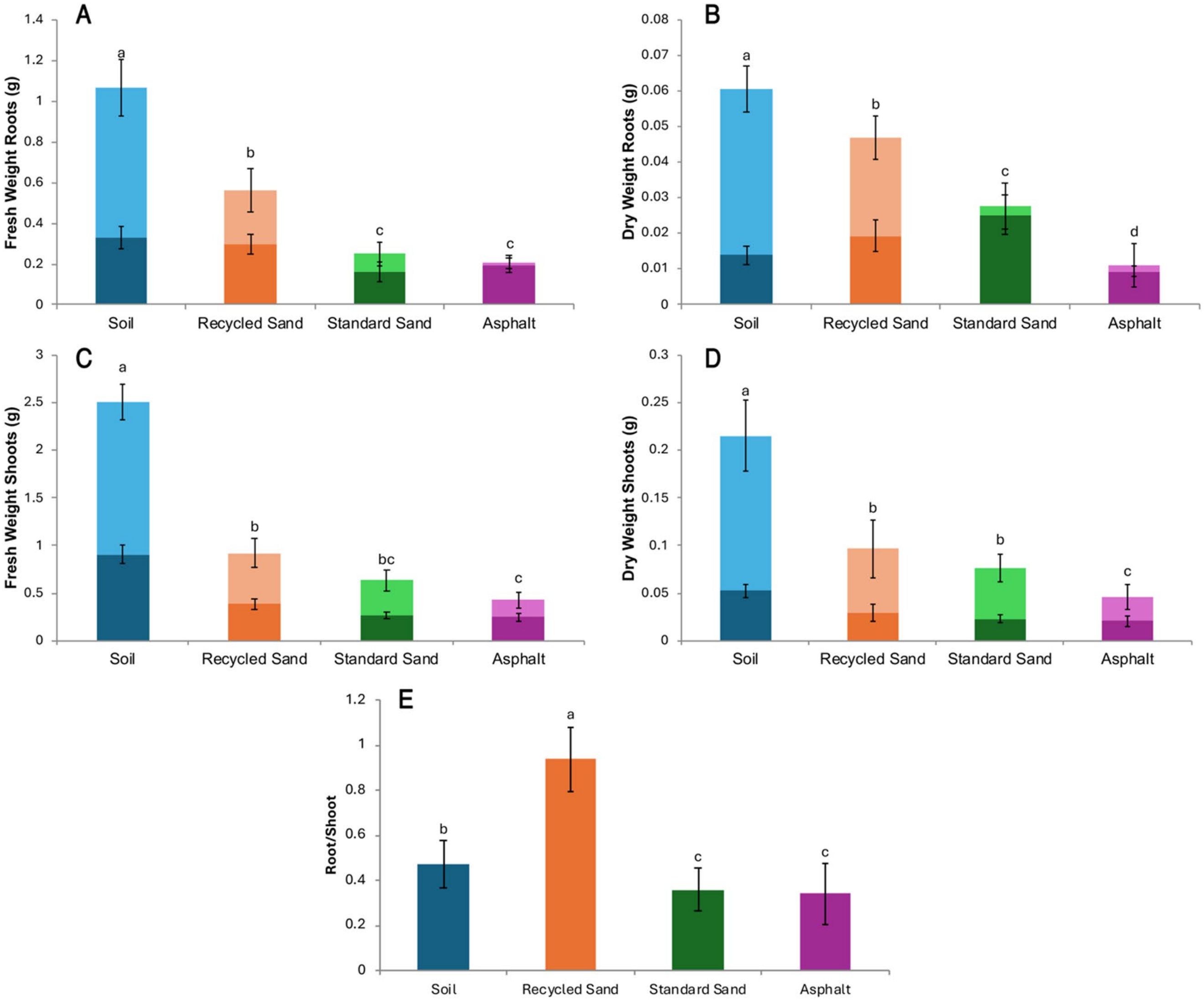

Fig. 4 Root and shoot biomass accumulation of *Borago officinalis* seedlings grown on four different substrates. Panels show fresh weight of roots (**A**), dry weight of roots (**B**), fresh weight of shoots (**C**), dry weight of shoots (**D**), and root/shoot ratio (**E**). Bars with two colours represent 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

Biomass accumulation

Substrate type had a strong influence on biomass accumulation over time (Fig. 4). Fresh and dry weights of both roots (Fig. 4A and B) and shoots (Fig. 4C and D) 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 and B).

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).

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).

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,

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

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

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).

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 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 sands 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.

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Data availability Data generated and analyzed during the current study are available from the corresponding author upon request.

Declarations

Competing interests The authors declare no competing interests.

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