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# Incorporating reclaimed asphalt pavement into constructed urban soils: Effects on plant growth and nitrogen-cycling bacteria

Bianca Rompato[a,b,1], Pasquale Napoletano[a,1], Maria Teresa Ceccherini[a], Giacomo Certini[a,*]

[a] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI), Università degli Studi di Firenze, Firenze 50144, Italy
[b] CRETUS, Departamento de Edafoloxía y Química Agrícola, Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain

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ABSTRACT

*Purpose:* This study explores the feasibility of using removed and crushed asphalt pavement mixed with compost from household organic waste to construct soil for green spaces, aiming to promote the recycling of wastes in urban contexts.

*Methods:* We conducted a pot experiment testing different treatments with varying proportions of asphalt pavement (10%, 25%, or 50% v/v), a fixed amount of compost (5% v/v), and excavated urban soil making up the remainder. A mixture of grasses commonly used in urban green infrastructure was sown and monitored for vegetative growth. Potential phytotoxicity of the constructed soils was assessed through a germination test, while the release of heavy metals and polycyclic aromatic hydrocarbons (PAHs) was evaluated by analysing both the solid phase and its leachate. The abundance of functional nitrogen-cycling bacterial communities in the constructed soils was characterised using quantitative PCR.

*Results:* The combined addition of asphalt and compost did not impair soil properties, and the treatment with an intermediate proportion of asphalt exhibited satisfactory plant growth without exceeding legal limits for heavy metals and PAHs. Furthermore, substrates containing asphalt effectively supported the development of key bacterial populations involved in the nitrogen cycle.

*Conclusions:* This study demonstrates the potential for disused asphalt to be repurposed on-site as a component in constructed urban soils for greening rather than being discarded as landfill waste, thereby aligning with the UN Sustainable Development Goal 11 (Sustainable Cities and Communities) of the 2030 Agenda. However, further studies in other contexts and over a longer period are needed to fully demonstrate that this practice does not have significant negative consequences on herbaceous vegetation growth, soil health, or public safety.

## 1. Introduction

Most of the world's population has resided in cities for some time now, and the trend continues to rise. In recent years, urban surface area has been growing by 2–3% each year (Zhao et al. 2022). This trend has significant environmental and social implications worldwide, calling for a re-evaluation and replanning of our cities to make them as livable as possible (Javed et al. 2022). In this context, urban green areas and their soils are crucial for delivering a wide range of ecosystem services, including microclimate regulation, pollutant and dust removal, and the provision of recreational and aesthetic benefits (Anguluri and Narayanan, 2017; O'Riordan et al., 2021). Consequently, the implementation of green infrastructures and urban soil restoration, through practices like decompaction and organic matter enrichment to enhance soil fertility, is increasingly being pursued (Deeb et al. 2020; Fite et al. 2011; Rompato et al. 2025a). Soil desealing is recognized as a vital approach for creating new green spaces in cities, helping to mitigate the impacts of extreme weather events (e.g., downpours and heatwaves) while enhancing the daily quality of life for urban populations (Vieillard et al. 2024). Although some authors – e.g., Renella (2020); Ugolini et al. (2020); Maienza et al. (2021) – have reported that desealed soils can rapidly improve their physical, chemical, and biological fertility through colonization by volunteer plants or the planting of appropriate species, achieving complete recovery remains a challenging process for most of these sites. To counter the negative effects of prolonged soil sealing, unsealed soils require amendments that improve drainage, fertility, and

* Corresponding author.
*E-mail address:* giacomo.certini@unifi.it (G. Certini).
[1] The authors contributed equally to this work

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microbial activity to foster effective rehabilitation. Since the application of circular economy principles is a key aspect of sustainable urban planning, it is beneficial to reuse on-site resources and repurpose waste materials. Constructing functional soils (Technosols) from these materials can further enhance the environmental sustainability of urban areas (Fabbri et al. 2021).

The use of compost from urban organic waste to construct soils for urban greening and to enhance water retention, nutrient availability, and microbial activity is well documented (e.g., Vitale et al. 2024; Pruvost et al. 2025). In contrast, the use of inorganic fractions of urban waste—such as inert building materials and asphalt pavement removed from roads and parking lots—for the same purpose is still seldom considered, and such materials often end up in landfills. Although a few studies demonstrate that this practice has great potential for supporting urban sustainability (Rompato et al. 2025b; Mascetti et al. 2025; Mastrolonardo et al. 2026), this remains an underexplored area of research, and aspects concerning soil ecology and soil health are still insufficiently understood. Asphalt pavement, a consolidated mixture of aggregates (gravel, sand, filler) and a bituminous binder, has significant environmental impacts throughout its lifecycle. When it cannot be reused for new pavement layers, as in the case of aged mixtures, its disposal poses challenges due to the large volumes involved and its complex composition, which includes potentially hazardous polycyclic aromatic hydrocarbons (PAHs) (Azadgoleh et al. 2022) and various heavy metals, the latter often resulting from vehicle traffic (Bäckström et al., 2004). Nevertheless, in a pot experiment, Rompato et al. (2025b) showed that mixing up to 30% crushed reclaimed asphalt pavement (RAP) from roadworks with excavated urban soil and compost did not inhibit the growth of herbaceous species or soil microbial activity. Furthermore, this mixture did not lead to the release of heavy metals or PAHs in leachates over short timescales. A related study by Mikajlo et al. (2025) demonstrated that the effectiveness of this approach depends primarily on the proportions of RAP and compost used in the Technosol formulation. Therefore, it is necessary to test various types and dosages of asphalt to better understand its impact on soil processes, plant responses, and environmental sustainability.

This study assesses the potential for constructing urban soils by incorporating increasing doses of RAP, specifically 10%, 25%, and 50% by volume. Based on the aforementioned research, we hypothesized that the lowest proportion (AS−10) would not cause substantial negative effects on soil chemistry, plant performance, and nitrogen-cycling microbial populations, while the highest RAP proportion (AS−50) may represent a hazardous excess. To test this, a pot experiment was conducted using a mixture of excavated urban soil, urban waste compost, and crushed RAP. The experiment monitored the growth of a consortium of herbaceous species while evaluating potential risks to human and environmental health from the release of heavy metals and PAHs. In addition, the impact of RAP on the constructed soils was evaluated by characterizing key physicochemical properties and analyzing the main functional groups of soil bacteria involved in the nitrogen cycle. Nitrogen was selected as a focus because it is a critical plant macronutrient, and its associated microbial taxa are highly sensitive, serving as effective early indicators of environmental change.

This research integrates soil-plant dynamics with a comprehensive evaluation of broader environmental impacts. Specifically, it aims to: *i*) determine the maximum RAP threshold for urban greening that avoids negative effects on seed germination and herbaceous growth, and *ii*) assess the extent to which nitrogen-cycling microorganisms are influenced by varying RAP concentrations. By combining substrate chemistry, leachate contaminant analysis, plant growth, and quantitative polymerase chain reaction (qPCR), this study employs an integrated design that is still relatively rare among RAP-based soil studies conducted under outdoor experimental conditions.

## 2. Material and methods

### 2.1. Experimental design and substrate composition

The experimental design followed a randomized block scheme with four treatments and three replicates per treatment. Twelve mesocosms were constructed using plastic cylinders (Figure S1), each with a volume of 3.08 dm$^3$ and dimensions of 20 cm in height and 14 cm in diameter. To prevent the loss of soil particles, the bottom of each cylinder was covered with a mesh and sealed with a perforated plastic base, allowing leachate to flow into a 10-cm-high collection tank positioned beneath the mesocosm.

The substrates for plant growth consisted of various mixtures of excavated urban soil, RAP, and compost. The soil and RAP were provided by Prato Scavi S.r.l., a local contractor specializing in urban excavation and infrastructure development, while the compost was sourced from a composting plant (Alia Servizi Ambientali; https://plures.aliaserviziambientali.it/it-it/notizie/2023/ottobre/impianto-faltona) that processes organic and green waste collected through municipal door-to-door programs. Prior to the experiment, the three materials were sieved to < 1 cm and mixed in the following proportions by volume:

- **AS−0**: 95% soil, 5% compost
- **AS−10**: 85% soil, 5% compost, 10% RAP
- **AS−25**: 70% soil, 5% compost, 25% RAP
- **AS−50**: 45% soil, 5% compost, 50% RAP

.

Hence, all treatments mantained a constant 5% compost content, with the remaining proportion consisting of varying ratios of soil and RAP.

The mesocosms were sown with 0.4 g of a commercial seed mix consisting of *Lolium perenne* L. (50% by seed weight), *Festuca rubra* L. (25%), and *Poa pratensis* L. (25%), which are species commonly used for urban lawns. The mesocosms were then placed on a covered outdoor workbench. The trial was conducted from July 2024 and lasted for 87 days after sowing (DAS). Throughout the experiment, the grass was cultivated under natural light and ambient temperature conditions. Daily temperatures were obtained from Consorzio LaMMA (Laboratorio di Monitoraggio e Modellistica Ambientale, https://www.lamma.toscana.it/clima-e-energia/climatologia/report-mensili-toscana) and are presented in Figure S2. A standardized watering schedule was followed, with 200 mL of deionized water applied to each mesocosm every two days. No fertilizers or pesticides were used during the trial.

### 2.2. Sampling and analysis

Aboveground biomass data were collected at 27, 48, and 87 days after sowing (DAS), with the final sampling coinciding with the end of the trial. Grass height was measured manually using a ruler at each time point and expressed as the mean across the three sampling times. At the same intervals, the grass was harvested and oven-dried at 60 °C until a constant weight was achieved. The dry weight obtained from each sampling was then summed, normalized to the surface area of the mesocosm, and expressed as grams per square meter (g m$^{-2}$).

Destructive soil sampling was performed on DAS 87. A representative aliquot of known volume was oven-dried at 105 °C to a constant weight to determine the dry mass and calculate the bulk density (BD). The remaining soil was air-dried; a portion was used to determine the water holding capacity (WHC) via the funnel method described by Bernard (1963), while the remainder was sieved through a 2 mm mesh for further analysis. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 (w/v) soil-to-deionized water suspension using an XS PC8 pH meter. Cation exchange capacity (CEC) was determined using the barium chloride–triethanolamine method. Total nitrogen (N tot) was quantified using an elemental analyzer (NC Soil Analyzer, Thermo Fisher, Flash EA 1112 series) after pulverizing the sample. Organic carbon content (C org) was calculated by multiplying the loss-on-ignition (in a muffle furnace for at least four hours at 460 °C) by the Van Bemmelen factor of 0.58.

Heavy metals (Cu, Cd, Ni, Pb, and Zn) were measured in the soil at DAS 87 (the end of the experiment) and in leachates collected on DAS 1 and 87. For leachate collection, the mesocosms were positioned above collection tanks to facilitate drainage. They were initially watered with 700 mL and subsequently with an additional 800 mL. Twenty-four hours after the first watering, the volume of leachate was measured, and an aliquot was collected for heavy metal analysis. Measurements were carried out on diethylenetriaminepentaacetic acid (DTPA) soil extract—obtained according to the ISO 14870:2001 method—by inductively coupled plasma optical emission spectrometry (ICP-OES, by an iCAP 7000 Plus series, Thermo Scientific). Detection limits were 0.001 mg $L^{-1}$ for Cu and Ni and 0.0001 mg $L^{-1}$ for Zn. DTPA was preferred as an extractant over ethylenediaminetetraacetic acid (EDTA) due to the alkaline nature of the soils (pH > 6.5; Feng et al., 2005). The metal concentrations detected in the leachate were expressed per cubic decimeter of soil to facilitate comparison among treatments.

Thirteen main PAHs (see list in Table 2) were determined in both the original crushed asphalt and in all soil treatments, following EPA methods 3550 C (https://www.epa.gov/sites/default/files/2015–12/documents/3550 c.pdf) and 8270E (https://www.epa.gov/sites/default/files/2020–10/documents/method_8270e_update_vi_06−2018_0.pdf). The analysis was performed using an Agilent 7890 GC coupled with an Agilent 7000 Rev D triple quadrupole mass spectrometer. An ISO 17034-compliant analytical reference standard mixture—PAH Mixture 932 at 200 µg/mL in dichloromethane—was used for calibration and quantification.

### 2.3. Germination test for phytotoxicity

A germination test was conducted using watercress (*Lepidium sativum* L.) seeds, following the UNICHIM 1651:2003 method, to evaluate the potential phytotoxicity of the substrates (Figure S3a). For each treatment and replicate (12 dishes in total), ten seeds were placed in a Petri dish. Each dish was treated with 3 mL of an aqueous extract prepared from the respective substrate by hydrating 200 g of dry sample to 85% WHC, stirring for 2 h, and allowing it to settle by gravity for 30 min before filtration.

A control was prepared using distilled water. The dishes were incubated in the dark at 25 ± 2 °C for 72 h. At the end of the exposure period, the number of germinated seeds and the root length of each seedling were recorded (Figure S3b). The effects on germination and root elongation were expressed as the germination index (GI), according to Eq. 1, as proposed by Zucconi et al. (1981):

$$GI\% = \frac{(G_T * L_T)}{(G_c * L_c)} * 100 \quad (1)$$

where $G_T$ and $G_c$ are the mean values of germinated seeds for the treatment and the control, respectively, while $L_T$ and $L_c$ correspond to the mean root lengths for the treatment and the control.

### 2.4. DNA extraction and quantitative PCR (qPCR) analysis

The functional groups of the soil bacterial community involved in the nitrogen cycle were studied through DNA extraction and subsequent amplification of specific genes by qPCR. Total soil DNA was extracted using a FastDNA™ SPIN Kit for Soil (MP Biomedicals), as described by Ceccherini et al. (2007). DNA purity was evaluated using a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific) by measuring absorbance ratios at 260/280 nm and 260/230 nm to assess protein and organic or salt contamination, respectively. DNA quality was assessed by 1% TAE agarose gel electrophoresis, stained with MIDORI Green Advance (5 µL per 100 mL), and run at 100 V for 60 min. Specific primer sets (Table S1) were used to quantify gene sequences of total eubacteria (*16S* rRNA), as well as nitrogen-fixing (*nifH*), ammonia-oxidizing (*amoA*), and denitrifying (*nirK*) microorganisms in each sample. qPCR reactions were carried out in a 25 µL mixture containing 1 × iTaq™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, CA), 10 µM of each primer, 50 ng of template DNA, and sterile double-distilled water ($ddH_2O$). Melting curve analysis was performed from 55 to 95 °C, with 0.5 °C increments every 5 s. Tenfold serial dilutions of positive controls were used to construct standard curves on a CFX Connect Real-Time PCR detection system (Bio-Rad). The amplification efficiency, coefficient of determination, and dynamic range of each gene standard curve are reported in Table S2. The qPCR cycle parameters were consistent across all targeted genes (Table S3), except for the annealing temperatures specified in Table S2 for each primer pair. The gene copy numbers—which do not account for the number of individuals—were normalized per gram of soil to allow comparisons across treatments and functional groups, according to Eq. 2 (Lee et al. 2008):

Gene copy number $g^{-1}$ soil = $6.02 \times 10^{23}$(copies $mol^{-1}$) x fragment amount (ng $g^{-1}$ of soil) / fragment length (bp) x ($1{\times}10^9$) x 660 (g $mol^{-1}$ $bp^{-1}$) (2)

### 2.5. Statistical analysis

All soil variables across the different treatments were analyzed in triplicate (n = 3), while leachate chemistry and plant responses were evaluated at multiple time points (n = 3 replicates x 2 sampling times). Results are expressed as mean ± standard deviation and presented on their original scale (untransformed).

Statistical analysis began with the Shapiro–Wilk test to assess the normality of the data ($p < 0.05$). To meet the assumptions of normality, reciprocal transformations were applied to BD, organic C, C/N ratio, EC, Ni at DAS 1, and *nif*H abundance. The homogeneity of variances was evaluated using Levene's test ($p < 0.05$). Subsequently, a one-way Analysis of Variance (ANOVA) followed by Tukey's honestly significant difference (HSD) *post hoc* test was used to identify statistically significant differences among the treatments ($p < 0.05$). All variables were tested separately, including leachates at different time points.

Statistical analyses were performed using SigmaPlot 15.0 (Systat Software Inc., San Jose, CA, USA), while all graphs were generated using OriginPro 2025 (OriginLab Corporation, Northampton, MA, USA).

## 3. Results

### 3.1. Soil properties

The compost characterization, as provided by the manufacturer, is reported in Table S4, while the measured substrate variables are shown in Table 1. Statistical analysis indicated that the BD was consistent between AS−0 and AS−10, but significantly higher in AS−50 (1.3 g $cm^{-3}$). Regarding WHC, a progressive and significant decrease was observed as the asphalt content increased, falling from 55.9% (AS−0) to 39.5% (AS−50). Both C org and the C/N ratio increased with higher asphalt percentages. Specifically, AS−50 contained 51% more C org than AS−0 (2.5%), and the C/N ratio was markedly higher in AS−25 (23.9) compared to AS−0 (16.7). Conversely, N tot showed minimal variation across treatments, maintaining values around 0.1% in all cases. AS−0 and AS−10 had similar C/N ratios and differed from AS−25 and AS−50, which exhibited comparable elevated values. No statistically significant differences were observed for CEC, with values ranging from 24 cmol(+) $kg^{-1}$ in AS−0 to 20.1 cmol(+) $kg^{-1}$ in AS−50. EC varied across treatments: AS−25 and AS−50 showed the lowest (251 µS

**Table 1**
Physicochemical properties of the experimental substrates, measured at the end of the experiment, 87 days after sowing (DAS). Values are presented as mean ± standard deviation (n = 3). Means within the same column followed by different letters are significantly different (p < 0.05) based on Tukey's HSD test performed on transformed data.

| Treatment | BD | WHC | C org | N tot | C/N | CEC | EC | pH |
|---|---|---|---|---|---|---|---|---|
| | g cm$^{-3}$ | % | % | % | | cmol kg$^{-1}$ | µS cm$^{-1}$ | |
| AS−0 | 1.14 ± 0.01 b | 55.9 ± 1.1 a | 2.5 ± 0.1 b | 0.1 ± 0.0 a | 16.7 ± 1.0 b | 24.0 ± 3.6 a | 283 ± 21.9 b | 8.1 ± 0.0 a |
| AS−10 | 1.13 ± 0.03 b | 49.6 ± 0.7 b | 2.8 ± 0.0 b | 0.1 ± 0.0 a | 18.8 ± 0.2 b | 21.4 ± 1.5 a | 363 ± 122 ab | 8.1 ± 0.2 a |
| AS−25 | 1.21 ± 0.01 ab | 43.7 ± 2.2 c | 3.6 ± 0.2 a | 0.1 ± 0.0 a | 23.9 ± 0.7 a | 21.8 ± 2.0 a | 251 ± 47.8 b | 8.3 ± 0.0 a |
| AS−50 | 1.30 ± 0.11 a | 39.5 ± 0.8 d | 3.7 ± 0.1 a | 0.2 ± 0.0 a | 22.7 ± 1.7 a | 20.1 ± 2.9 a | 414 ± 87.2 a | 8.2 ± 0.2 a |

Legend: BD = bulk density; WHC = water holding capacity; C org = organic carbon; N tot = total nitrogen; C/N = organic carbon to total nitrogen ratio; CEC = cation exchange capacity; EC = electrical conductivity. Treatments: AS−0 (95% excavated soil, 5% compost); AS−10 (85% soil, 5% compost, 10% RAP); AS−25 (70% soil, 5% compost, 25% RAP); and AS−50 (45% soil, 5% compost, 50% RAP).

cm$^{-1}$) and highest (414 µS cm$^{-1}$) values, respectively, while AS−0 and AS−10 showed intermediate values. The pH remained in the alkaline range (8.1–8.3) with no significant variation across treatments.

### *3.2. Heavy metals, polycyclic aromatic hydrocarbons, and vegetation traits*

The extraction of metals from the soil with DTPA at the end of the experiment (on DAS 87) yielded appreciable values only for Cu and Ni. Mean concentrations ranged from 116.4 to 144.7 µg dm$^{-3}$ of soil for the former and from 9.2 to 12.3 µg dm$^{-3}$ for the latter, with no statistically significant differences between treatments.

In the leachate, Pb and Cd were below the detection limit on both DAS 1 and 87, while Cu was consistently more abundant than Ni and Zn, though all occurred in relatively low quantities. The amounts of Cu released per cubic decimeter of substrate showed an increasing trend with increasing asphalt content on both sampling dates; however, only AS−0 and AS−50 differed significantly from each other (Fig. 1a). Leached Ni ranged between 4 and 8 µg dm$^{-3}$ of substrate across treatments and was significantly lower in the control than in both AS−25 and AS−50 on DAS 1, whereas no significant differences among treatments were observed on DAS 87 (Fig. 1b). Finally, no significant differences among treatments were found for Zn on either sampling date, with values clustering around 4 µg dm$^{-3}$ of soil (Fig. 1c).

PAH concentrations for the RAP and all soil treatments are summarized in Table 2. In the AS−0 treatment (the asphalt-free control), all PAHs were below detection limits (<0.005 mg L$^{-1}$). Conversely, in the treatments containing asphalt, extractable PAHs were detected, albeit at low concentrations. The RAP itself released PAHs—particularly benzo[*e*]pyrene, benzo[*g*,*h*,*i*]perylene, and chrysene—although in lower amounts than expected. The substrate with the highest asphalt content (AS−50) exhibited notably higher concentrations of benzo[*b*]fluoranthene, benzo[*g*,*h*,*i*]perylene, chrysene, and pyrene compared to AS−25 and AS−10. However, with the exception of a minor exceedance for benzo[*g*,*h*,*i*]perylene, all other measured PAHs remained well below the Italian legal thresholds for soils in residential and green areas (D.Lgs. 152/2006), as detailed in Table 2.

Average plant height, measured at 27, 48, and 87 DAS, was approximately 23 cm and did not differ significantly among treatments (Fig. 1e). In contrast, both the cumulative grass biomass (measured at

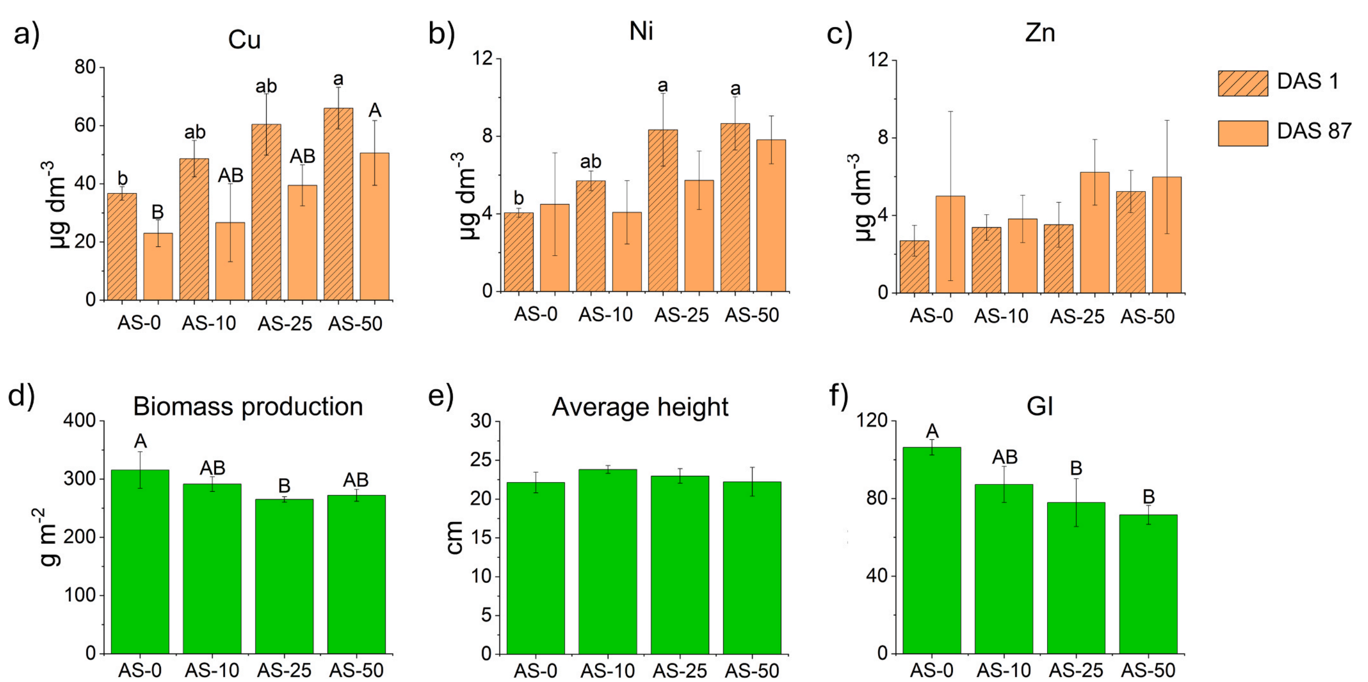


**Fig. 1.** Leached heavy metals and plant growth characteristics. (a) Cu, (b) Ni, and (c) Zn released in leachates (expressed as µg dm$^{-3}$ of soil) collected at 1 and 87 days after sowing (DAS 1 and DAS 87); (d) cumulative biomass production at DAS 87; (e) average plant height (DAS 27, 48, and 87); and (f) germination index (GI) across treatments (AS−0 = 95% excavated soil, 5% compost; AS−10 = 85% excavated soil, 5% compost, 10% RAP; AS−25 = 70% excavated soil, 5% compost, 25% RAP; AS−50 = 45% soil, 5% compost, 50% RAP). Values are presented as mean ± standard deviation (n = 3). For metals, different lowercase letters indicate statistically significant differences among the treatments at DAS 1, while different uppercase letters indicate significant differences at DAS 87 (Tukey HSD test, $p < 0.05$). Statistical differences in leachates were tested separately, DAS 1 and DAS 87 respectively.

**Table 2**
Extractable PAH concentrations (mg $kg^{-1}$) in experimental substrates and crushed removed asphalt pavement (RAP). Regulatory limits for urban park soils according to Italian legislation (D.Lgs. 152/2006) are provided for comparison. All values refer to the fine earth (< 2 mm).

| Polycyclic Aromatic Hydrocarbon (PAH) | AS−0 | AS−10 | AS−25 | AS−50 | RAP | Legal limit in Italy |
|---|---|---|---|---|---|---|
| Benz[*a*] anthracene | - | - | 0.007 ± 0.003 | 0.015 ± 0.008 | - | 0.5 |
| Benzo[*a*] pyrene | - | 0.008 ± 0.004 | 0.026 ± 0.012 | 0.061 ± 0.027 | 0.067 ± 0.034 | 0.1 |
| Benzo[*b*] fluoranthene | - | - | 0.014 ± 0.007 | 0.031 ± 0.013 | - | 0.5 |
| Benzo[*g,h,i*] perylene | - | 0.012 ± 0.006 | 0.054 ± 0.03 | 0.122 ± 0.067 | 0.082 ± 0.042 | 0.1 |
| Benzo[*k*] fluoranthene | - | - | - | 0.009 ± 0.005 | - | 0.5 |
| Chrysene | - | 0.009 ± 0.005 | 0.024 ± 0.012 | 0.051 ± 0.022 | 0.062 ± 0.032 | 5 |
| Dibenzo[a.e] pyrene | - | - | 0.006 ± 0.003 | 0.013 ± 0.007 | - | 0.1 |
| Dibenzo[a.h] anthracene | - | - | - | 0.007 ± 0.004 | - | 0.1 |
| Dibenzo[a.h] pyrene | - | - | - | - | - | 0.1 |
| Dibenzo[a.i] pyrene | - | - | - | 0.006 ± 0.003 | - | 0.1 |
| Dibenzo[a.l] pyrene | - | - | - | - | - | 0.1 |
| Indeno[1.2.3-cd]pyrene | - | - | 0.01 ± 0.005 | 0.022 ± 0.011 | - | 0.1 |
| Pyrene | - | 0.008 ± 0.004 | 0.016 ± 0.008 | 0.042 ± 0.018 | - | 5 |

Legend: AS−0 = 95% excavated soil and 5% compost; AS−10 = 85% excavated soil, 5% compost, and 10% RAP; AS−25 = 70% excavated soil, 5% compost, and 25% RAP; AS−50 = 45% soil, 5% compost, and 50% RAP; - = not detected.

27, 48, and 87 DAS; Fig. 1d) and the GI for *L. sativum* (Fig. 1f) showed a declining trend with increasing asphalt content. The AS−0 treatment recorded the highest mean values for both biomass (315.6 g $m^{-2}$) and GI (106.4%). However, these values were significantly different only from AS−25 for biomass, and from AS−25 and AS−50 for GI, indicating only a partial progressive decrease along the gradient of increasing asphalt doses.

### *3.3. Molecular data*

In terms of nitrogen-fixing *nifH* gene copy number ( Fig. 2a), AS−0 showed the highest values and AS−10 the lowest, with $1.10 \times 10^8$ and $7.36 \times 10^7$ copies $g^{-1}$ soil, respectively. Intermediate values were recorded for AS−25 and AS−50, which were 20.1% and 30.1% lower than AS−0, respectively.

For the ammonia oxidizer *amo*A gene copy number (Fig. 2b), the highest mean abundance was observed in AS−10 ($8.36 \times 10^7$ copies $g^{-1}$ soil), although this was not significantly different from AS−0 ($3.01 \times 10^7$), AS−25 ($4.37 \times 10^7$), or AS−50 ($3.79 \times 10^7$).

For the denitrifier *nirK* gene copy number (Fig. 2c), a clear increase from AS−0 to AS−50 was observed. The AS−50 treatment displayed the highest value, with $1.13 \times 10^{10}$ copies $g^{-1}$ soil, which was statistically different from AS−0 ($6.02 \times 10^9$) but comparable to AS−25 ($1.01 \times 10^{10}$) and AS−10 ($7.91 \times 10^{9}$).

A progressive increase in total DNA yield—an estimate of total microbial biomass (prokaryotic and eukaryotic)—was observed across the treatments (Figure S4). However, the bacterial *16S r*RNA gene copy number (Fig. 2d) did not change significantly with increasing amounts of RAP, reaching the highest mean value of $5.80 \times 10^9$ copies $g^{-1}$ soil in the AS−50 treatment.

## 4. Discussion

This research provides novel insights into the use of RAP within urban green infrastructure. Such a holistic approach, combining plant, physical, chemical, and microbial indicators under outdoor mesocosm conditions, is rarely applied in RAP-based soil studies. These findings contribute to a better understanding of the potential and limitations of RAP as a substrate component, highlighting both its environmental implications and its suitability for vegetation establishment.

### *4.1. Soil properties, vegetation traits, and potential pollution*

Our starting hypothesis—that moderate asphalt incorporation into excavated urban soil, when amended with compost, does not significantly impair selected soil properties or plant growth—was largely supported by this study, aligning with recent findings by Mikajlo et al. (2025) and Rompato et al. (2025b). RAP did not significantly increase substrate bulk density, except in the AS−50 treatment. The observed decrease in WHC with higher RAP percentages can be attributed to the hydrophobic nature and non-porous structure of asphalt, which limit water retention. While this increased drainage could have positive implications for urban environments by contributing to stormwater runoff mitigation (Bhattacharya, 2021; Morard and Silvestre, 1996), it may simultaneously accelerate the leaching of nutrients, such as nitrate ($NO_3^-$), into the groundwater, representing a potential drawback for urban greening.

Enhanced nitrogen mobility over longer timescales than those investigated here may diminish soil fertility and limit the growth of herbaceous species typically used in green infrastructure, ultimately compromising ecosystem services such as urban cooling, stormwater regulation, and pollution mitigation. Additionally, the transport of nitrate-rich leachates into groundwater and surface waters can degrade water quality and trigger eutrophication, resulting in cascading effects on ecological functioning. These risks highlight the importance of integrating nutrient-retentive management practices when employing RAP-based substrates.

Moreover, the increase in organic carbon observed with higher RAP proportions is likely linked to the inherently high carbon content of bitumen, which partially volatilized during the loss-on-ignition process. This was accompanied by an increase in the C/N ratio, reflecting the highly condensed and recalcitrant nature of bitumen-derived carbon and its limited association with nitrogen. Importantly, the CEC remained stable across treatments; this is a positive finding, as a reduction in CEC could have undermined the substrate's capacity to retain nutrients—an essential factor for plant growth in urban greening applications. The effect on pH was also modest, with no significant shifts across treatments. However, the highest dose of RAP (AS−50) induced soil salinization.

The limited impact of RAP on key chemical parameters, such as CEC, EC, and pH, suggests that RAP-based substrates offer chemical stability compatible with vegetation, representing a promising opportunity for urban green infrastructure. However, the increase in the C/N ratio indicates the presence of highly stable organic carbon fractions with negligible nutritional value, suggesting that supplemental fertilization or amendments may be necessary to sustain long-term plant productivity.

In this framework, the AS−10 mixture appears to be the most suitable combination, successfully integrating soil physicochemical properties with favorable plant responses. Notably, even the AS−50 mixture does not appear particularly detrimental to soil–plant dynamics, despite its higher salinity and bulk density.

Overall, these findings align with those reported by Spreadbury et al. (2021), who critically reviewed the potential environmental risks associated with the incorporation of asphalt into soils. The authors concluded that asphalt does not pose a significant concern for pollutant release, particularly regarding heavy metals and PAHs. Indeed, Legret

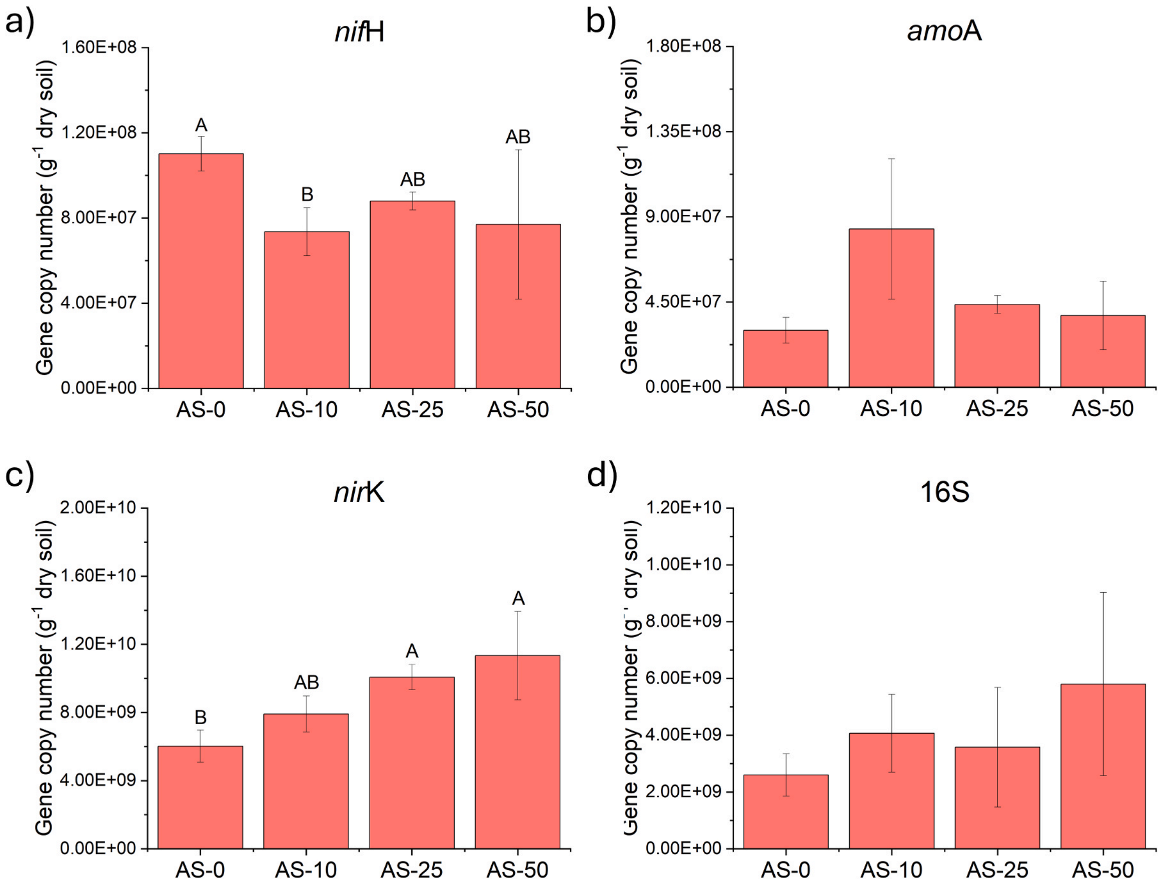


**Fig. 2.** Gene copy numbers for the functional genes and the *16S* rRNA marker across the treatments. (a) *nifH*, (b) *amoA*, (c) *nirK*, and (d) *16S* rRNA marker at DAS 87. Treatments are: AS−0 = 95% excavated soil, 5% compost; AS−10 = 85% excavated soil, 5% compost, 10% RAP; AS−25 = 70% excavated soil, 5% compost, 25% RAP; AS−50 = 45% soil, 5% compost, 50% RAP. Values are presented as mean ± standard deviation (n = 3). Means with different letters are significantly different ($p < 0.05$) based on Tukey's HSD test performed on transformed data.

et al. (2005) had previously demonstrated, through batch tests and leaching experiments performed on core samples taken from two rebuilt road sections containing 10% and 20% of reclaimed asphalt pavement, that the leaching of selected heavy metals and polycyclic aromatic hydrocarbons (PAHs) was rather weak. Furthermore, the concentrations in solutions from batch leaching tests were generally below the European Union (EU) drinking water limit values.

In agreement, the concentrations of heavy metals and PAHs detected in all substrate leachates in this study were well below legal thresholds (EU 2006; Italian Republic, 2006), with the exception of a slight exceedance of benzo[*g*,*h*,*i*]perylene in AS−50, which may also reflect a historical accumulation from vehicular traffic and subsequent adsorption onto the asphalt surface prior to its excavation (Markiewicz et al., 2017; Fang et al., 2005). This outcome is consistent with the known low mobility of PAHs in soil environments (Garcìa Frutos et al., 2011) and supports the use of excavated asphalt in constructed soils for urban greening as a safe and environmentally sound practice. These results provide reassurance regarding the absence of significant intrinsic releases from the asphalt and the low risk of contamination arising from environmental exposure.

Nevertheless, to ensure environmental safety, long-term monitoring would be advisable to evaluate potential delayed impacts. Indeed, the detection of PAHs such as benzo[*b*]fluoranthene, benzo[*g*,*h*,*i*]perylene, chrysene, and pyrene in the AS−50 treatment, though well below regulatory limits, raises the possibility that weathering over time may gradually release these compounds from the bitumen matrix (Peng et al. 2008). On the other hand, it is also known that microbial degradation is the primary pathway for PAHs, and several bacterial species capable of promoting such processes are frequently isolated from contaminated soil or sediments (Haritash and Kaushik, 2009). Regardless, compared to other treatments, AS−50 exhibited a wider range of PAH classes, indicating a higher potential for long-term impact on environmental quality in urban ecosystems.

Increasing RAP content tended to reduce the germination index, likely due to a relative decrease in nutrient availability. However, the observed values ranged from "no phytotoxicity" (AS−10) to "moderate phytotoxicity" (AS−50), according to the classification of Zucconi et al. (1981). In this framework, GI < 50% indicates high phytotoxicity, a GI between 50% and 80% indicates moderate to low phytotoxicity, and GI > 80% indicates no phytotoxicity. Importantly, this initial reduction in the germination index did not translate into significant differences in average plant height or biomass production, suggesting that the impact on overall plant development remains limited, even at the 50% dose. Although the GI was obtained based on a dicotyledonous indicator (*Lepidium sativum*), while plant height and biomass production refer to a monocotyledonous grass, alignment with the scientific literature confirms that asphalt exerts selective and inhibitory pressure during early phenological stages (germination and early root elongation), while its long-term impact on overall vegetative development remains constrained by intrinsic plant resilience. Conversely, the incorporation of compost proved effective in promoting healthy plant growth and enhancing both the chemical and physical properties of the soil, as previously observed by Vitale et al. (2024) and Rompato et al. (2025b).

*4.2. Bacterial communities and nitrogen cycle populations*

Bacterial community measurements, such as gene abundance, are reliable indicators of how specific treatments affect soil ecosystems. When evaluating biogeochemical cycles, the targeted monitoring of functional genes is particularly effective. However, experiments measuring gene copy numbers reflect the standing bacterial community and its metabolic potential, rather than providing a direct measure of gene expression or enzymatic activity. As this study served as an initial screening of asphalt effects over a short 87-day mesocosm period, the nitrogen cycle was selected as the focal point due to its role in providing a critical plant nutrient. Nevertheless, pollutant dynamics over longer durations, as well as other nutrient cycles and gene expression levels, warrant further investigation.

Molecular quantification indicated that, on DAS 87, all targeted genes were present and that the asphalt incorporation influenced N-cycling bacterial populations. Specifically, the abundance of *16S* rRNA gene sequences exceeded those of *nifH* and *amoA* but was lower than that of *nirK*. This observation may be attributed to the high taxonomic diversity of denitrifiers, as the *nirK* gene is distributed across eubacteria, archaea, and fungi (Datta et al., 2020; Philippot and Hallin, 2005; Van Spanning et al., 2007). Furthermore, the *nirK* primers used in this study can detect all these microbial groups (Henry et al. 2004), potentially contributing to the high copy numbers observed. In particular, the denitrifying *nirK* gene sequences were two to three orders of magnitude more abundant than the nitrogen-fixing *nifH* and ammonia-oxidizing *amoA* gene sequences. However, only the populations represented by the *nifH* and *nirK* genes showed significant, though not drastic, decreases and increases, respectively. It is well established that $N_2$-fixers can adapt to environmental changes by modulating both their proliferation rates and metabolic activity (Zhang et al., 2025; Pulikova and Gorovtsov, 2022 ). Some $N_2$-fixers are also known to degrade organic contaminants, although their efficiency may vary depending on rhizospheric conditions and plant species (Muratova et al. 2003).

The slight increase in ammonia oxidizers at 10% RAP can be partially attributed to heterotrophic activity. Indeed, some microbial isolates are capable of oxidizing ammonia in the presence of organic substrates, including hydrocarbons present in bitumen-contaminated soils (Martikainen, 2022). Furthermore, genes encoding ammonia monooxygenase have been identified in heterotrophic genera such as *Pseudomonas*, *Corynebacterium*, and *Mycobacterium* (Daum et al. 1998; Muratova et al. 2003). This is consistent with the increase in *nirK* sequences, which may be due to the heterotrophic nature of many denitrifiers that allows them to thrive in soils enriched with organic matter or dissolved organic carbon (Bárta et al. 2010; Durand et al. 2024). Finally, the increase in BD observed in AS25 and AS50 (Table 1) could have induced microaerophilic conditions that favoured denitrifying microorganisms (Lipiec and Hatano, 2003; Mentges et al. 2016). However, the responses of denitrification-related genes (*nir*S, *nir*K, and *nos*Z) to such treatments can vary considerably (Philippot et al. 2007), underscoring the need for further targeted investigations, including the effects of compaction on denitrifying microorganisms in these types of constructed soils. Given that qPCR measures gene copy number rather than gene expression, these data do not directly quantify nitrogen transformation rates. However, the observed increase in the abundance of denitrifying bacteria suggests a greater potential for denitrification activity. Since varying asphalt concentrations seem to influence nitrogen metabolism, with higher doses specifically increasing the abundance of denitrifying sequences, from this perspective the AS−10 treatment (85% excavated soil, 5% compost, and 10% crushed RAP) appears to represent an optimal compromise for urban soil restoration.

## 5. Conclusions

This study demonstrates that incorporating reclaimed asphalt pavement (RAP) into constructed soils is a viable strategy for urban greening initiatives, such as vegetated roundabouts and traffic-dividing green spaces, while also supporting urban waste valorization. A combined assessment of plant growth, physicochemical soil properties, and bacterial gene indicators provides robust evidence that low RAP doses can be safely integrated into landscaping substrates. Our findings suggest that the incorporation of 10% (v/v) RAP offers an optimal balance between soil stability, nutrient retention, and plant performance. Additionally, qPCR analysis provides insights into the integrity of the nitrogen cycle. While higher RAP doses (up to 50%) remain a feasible option, as both heavy metal and PAH concentrations remained below Italian legal thresholds for residential and green area soils, they require careful management to mitigate negative impacts on nutrient availability, water retention, and the nitrogen cycle.

Overall, this research supports the sustainable use of RAP in urban greening, highlighting opportunities to reduce landfill disposal and improve resource efficiency, thereby contributing to progress toward UN Sustainable Development Goal 11 (Sustainable Cities and Communities) of the 2030 Agenda. The transferability of our findings to real-world green infrastructure may be limited by the small scale of the mesocosms, where root development, drainage, and microbial dynamics may differ from field conditions. Moreover, the absence of fertilizers and pesticides during the study may not reflect typical urban management practices. Therefore, long-term monitoring of RAP-based substrates under different conditions and across various urban contexts is recommended to ensure environmental safety, particularly regarding the potential slow release of polycyclic aromatic hydrocarbons (PAHs).

## CRediT authorship contribution statement

**Bianca Rompato:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Pasquale Napoletano:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Maria Teresa Ceccherini:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Giacomo Certini:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ufug.2026.129623.

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