SCUOLA DI DOTTORATO
UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

Department of Earth and Environmental Sciences (DISAT)

PhD program Chemical, Geological and Environmental Sciences Cycle XXXVIII

Curriculum in Environmental Sciences

Restoring Urban Soils After Desealing: Functional Recovery and Circular Reuse of Recycled Aggregates

Surname: Mascetti

Name: Gaia

Registration number: 834833

Tutor: Antonio Finizio

Supervisor: Chiara Ferré

Coordinator: Giovanni Marco Malusà

ACADEMIC YEAR 2024/2025

This PhD Thesis has been reviewed by:
Giacomo CERTINI, Università degli Studi di Firenze,
Gabriele BERETTA, Politecnico di Milano.

Table of Contents

General Abstract 7
CHAPTER 1 9
General Introduction 9
URBAN SOILS 10
The Issue of Soil Sealing 11
Environmental and Ecological Impacts 12
Policy Frameworks and Mitigation Strategies 13
DESEALING PRACTICES AND BENEFITS 14
Desealing and circularity: from demolition waste to constructed soils 15
RESEARCH GOALS AND THESIS OUTLINE 17
Bibliography 18
CHAPTER 2 21
Soil de-sealing and recycled aggregates application: one year of monitoring 21
INTRODUCTION 23
MATERIAL AND METHODS 26
Study Area and Experimental Design 26
Soil Sampling and Analyses 28
Leaching Test and Percolation Water 28
Bacterial Community Characterization 29
Data Processing and Analysis 29
RESULTS 31
Pedoclimatic Factors 31
Soil Temperature 31
Soil water content 31
Oxygen content in soil air 32
Soil Properties 32
Percolation Water 32
Bacterial Community Composition 33
Soil, pedoclimate and Bacterial Communities 33
DISCUSSION 36
De-sealing Effects on Pedoclimate Factors 36
Temporal changes in soil properties and RA effects 38
Bacterial community composition and evolution 39
CONCLUSIONS 41

Appendix A. Sensor Data Processing 43
Bibliography 44
Supplementary materials 47
CHAPTER 3 51
Restoring Urban Soils after Desealing: Effects of Recycled Aggregates, Compost, and Vegetation on Soil Properties and Microbial Communities 51
Abstract 52
INTRODUCTION 53
MATERIALS AND METHODS 54
Soil sampling and Analyses 56
Vegetation monitoring 57
Data Analysis 57
RESULTS 58
Short-term effects of Desealing and RA addition 58
Soil properties 58
Bacterial communities 59
Effects of the experimental treatments 62
Soil properties 62
Soil bacterial communities 63
Vegetation growth 66
DISCUSSION 68
Short-term effects of Desealing and RA addition 68
Experimental treatments 69
Soil properties 69
Bacterial communities 70
Vegetation growth 70
CONCLUSION 71
Bibliography 72
Supplementary materials 74
CHAPTER 4 78
Impacts of Recycled Concrete Aggregates and Reclaimed Asphalt Pavement on soil microbial activities and physicochemical properties: an indoor-mesocosm experiment 78
Abstract 79
INTRODUCTION 80
MATERIAL AND METHODS 81
Lufa Soil and Recycled Aggregates 81

Experimental design ... 82
Physical and Chemical Analyses ... 83
Assessment of Soil Microbial Activities ... 84
Enzymatic activities ... 84
Microbial basal respiration ... 84
Phytotoxicity tests ... 85
Statistical elaboration ... 86
RESULTS ... 87
Soil physical and chemical properties ... 87
Water Content, Compaction and Percolation ... 87
Soil pH and Conductivity ... 89
Soil Nutrient Content ... 90
Assessment of Microbial Activities ... 92
Enzymatic activities ... 92
Microbial basal respiration ... 93
Phytotoxicity tests ... 96
DISCUSSION ... 93
Effects on soil physicochemical properties ... 93
Microbial communities' responses ... 94
Phytotoxic effects ... 95
CONCLUSION ... 96
Bibliography ... 97
Supplementary materials ... 100
CHAPTER 5 ... 112
Growth responses of *Trifolium incarnatum* to increasing concentrations of concrete based recycled aggregates in soil ... 112
Abstract ... 113
INTRODUCTION ... 114
MATERIALS AND METHODS ... 115
Rhixoboxes Experimental Design ... 115
Sampling and Analysis ... 116
RESULTS ... 117
Soil Properties ... 117
T. incarnatum Growth and Biomass ... 117
DISCUSSION ... 120
Soil Properties ... 120
CONCLUSION ... 121

Bibliography.. 122
CHAPTER 6 124
General Conclusions.. 124
GENERAL CONCLUSIONS... 125
Acknowledgements 128

General Abstract

Urban soils play a fundamental role in sustaining biodiversity, regulating hydrological and biogeochemical cycles, and supporting vegetation growth. However, the increasing human pressure of the last century has led to a critical loss of essential soil functions in urban environments, impairing ecosystem stability and resilience. Among the various anthropogenic threats, soil sealing represents one of the most severe causes of soil degradation and land take in Europe.

This thesis investigates soil desealing (or depaving) as a tool for restoring urban soil functionality, and evaluates the environmental suitability of recycled aggregates (RAs) derived from construction and demolition waste (CDW) as soil constituent materials in desealed site, within a circular economy perspective. The work adopted a multidisciplinary approach combining field monitoring and controlled laboratory experiments to assess pedoclimatic dynamics, soil physicochemical properties, microbial communities, phytotoxicity, and plant responses. The overall aim is understanding early soil recovery processes after desealing and identifying potential constraints related to the incorporation of RAs in soil.

A field experiment conducted at Porto di Mare (Milan, Italy) assessed the combined effects of desealing, the application of concrete-based RAs, compost amendment, and vegetation establishment. Results showed that desealing effectively promotes soil restoration by improving pedoclimatic conditions, and that RAs, combined with compost addition and vegetation sowing, can support plant growth without inducing adverse effects on soil chemistry, bacterial communities, or percolation water quality. To further assess the effects and potential risks of RA use in soil, an indoor mesocosm experiment was carried out, under controlled conditions, at the University of Aveiro (Portugal). The experiment demonstrated that the incorporation of different RAs type, recycled concrete aggregates (RCA) and reclaimed asphalt pavement (RAP), induced material-specific changes in soil physicochemical properties, microbial activities, and early-stage plant development. Finally, a rhizobox experiment investigated the effects of increasing concrete-based RA concentration on plant development, revealing concentration-dependent responses,

that led to biomass reduction and adaptive shifts in resource allocation and root morphology.

Overall, this research provides new insights into soil functional recovery following desealing, as well as the environmental suitability of using RAs from demolition waste for soil reconstruction and urban greening. It demonstrates that combining desealing with circular reuse of these materials, carefully selected and applied, enhances both ecological recovery and resource efficiency.

CHAPTER 1

General Introduction

URBAN SOILS

Soils are fundamental to terrestrial life, as they provide and regulate most of the essential processes and functions that sustain ecosystems, including nutrient cycling, carbon storage, and water regulation [1]. But beyond their ecological importance, soils are strongly linked with human evolution, health and culture [2,3]. Indeed, urbanization and civilization processes have always been intrinsically linked to soils, whose availability and fertility – together with the access of fresh water – have historically guided the establishment and development of human settlements [2]. However, the same processes of urban growth that have depended on soils have also profoundly transformed them. Over the past century, rapid urbanization, intensive agricultural practices, industrial expansion, and infrastructure development have drastically modified land uses and soil properties, especially in urban and peri-urban areas [1,4].

As a result, soils in cities today are highly heterogeneous and exhibit complex spatial variability, with properties changing significantly even across small distances [2]. According to the gradient and typology of anthropic disturbance, they can range from pseudo-natural soils, still retaining some original structure, chemical properties and biological activity, to highly transformed soils and artificial substrates, that no longer retain the characteristics and functions of natural soils [5,6]

This heterogeneity complicates a comprehensive characterization of urban soils, as their distribution does not follow the soil–landscape relationships typical of natural environments. This poses significant challenges for their classification and management. Nonetheless, soil science and contemporary soil classification systems have recently recognized human activity as a soil-forming factor, leading to the introduction of new soil categories. In particular, the *World Reference Base for Soil Resources (WRB)* [7], classifies Anthrosols as soils deeply modified by prolonged and intensive agricultural use, while Technosols are characterized by the presence of artefacts – materials manufactured, transported, or strongly altered by humans – and are particularly prevalent in urban and industrial environments. These categories underscore the recognition of human activities as key geological and ecological drivers in the Anthropocene.

Despite their often altered and degraded characteristics, urban soils remain essential for sustaining ecosystem functions and supporting urban resilience [5]. Although their capacity to provide ecosystem services may be partially compromised, they still offer support for vegetation growth, regulate hydrological processes where permeability is maintained, and mitigate flood risk by enhancing water infiltration and storage. Additionally, they contribute to mitigate the urban heat island effect, and sustain biodiversity by providing habitat and food sources for a wide range of organisms [8,9].

In recent years, the increasing recognition of the ecological and social value of urban soils has driven the expansion of green infrastructure and nature-based solutions in cities worldwide. Initiatives such as urban parks, community gardens, green roofs, and vegetated corridors aim to restore ecosystem functions, enhance climate resilience, and improve human wellbeing. These green spaces provide opportunities for food production and biodiversity enhancement, while also promoting physical and mental health and social cohesion [2].

Effective and sustainable management of urban soils is crucial to align urban development with ecosystem integrity and to achieve the Sustainable Development Goal of inclusive, safe, resilient, and sustainable cities [10].

The Issue of Soil Sealing

In the last century, soil degradation across Europe was driven by both natural processes and the growing influence of human activities. Today, anthropogenic pressures are the primary drivers of soil degradation in many European regions [4]. They are driven by a wide range of complex and interconnected processes, including unsustainable management practices, industrial development, and land-use change, and despite their uneven spatial distribution, they may exert large-scale impacts. Among the main anthropogenic threats are erosion loss of soil organic matter, contamination, compaction, acidification, salinization, and sealing [4,11]. These pressures are most pronounced in urban and peri-urban areas, where they have led to the overexploitation, degradation, and, in some cases, irreversible loss of soil resources, impairing soil productivity and compromising their ecological and functional integrity [12,13]. The combined effect of these pressures generate a cumulative impact on soil systems and highlight the urgent need for sustainable land management and urban planning strategies.

Among the various human-induced forms of degradation, soil sealing is one of the most severe causes of soil degradation in Europe [14,15]. Artificial soil sealing is broadly defined as the removal of surface soil layers and/or the covering of soils with impermeable materials such as asphalt and concrete [15,16]. This process is primarily driven by the economic development and the demand for new infrastructures such as residential and commercial buildings, transport networks, and industrial facilities. Predominantly occurring at the edges of existing urban areas, it is closely associated with urbanization and represents the most severe form of land take, often involving the conversion of agricultural or natural land into built-up areas, resulting in the loss of fertile soils [16].

Soil sealing is particularly intense in densely populated and industrialized regions of Western and Central Europe, where artificial surfaces account for around 4% of the total land area in European Environment Agency (EEA) member states and exceed the 5% of the national territory in Malta, the Netherlands, Belgium, Germany, and Luxembourg member countries [4]. Recent assessments confirm that soil sealing and land take remain ongoing concerns: between 2012 and 2018, land-take in EU (including the United Kingdom) increased by 2.6%, with approximately 3,600 km^2 of new artificial surfaces [15]. Although the majority of soil sealing occurred in settlements, cropland was also significantly affected, with 1,383 km^2 of agricultural land sealed between 2006 and 2018 [17]. According to the most recent ISPRA report (2025), the net annual land take in 2024 in Italy reached 78.5 km^2, the highest value recorded in the past twelve years. Overall, artificial surfaces cover more than 21,500 km^2, corresponding to 7.17% of the national territory [18].

Environmental and Ecological Impacts

The environmental consequences of soil sealing are severe. The impermeable covers interrupt natural exchanges of water, gases, and energy between the soil and atmosphere, impairing soil functions essential for ecosystem stability.

It affects the soil's water regulation functions, leading to a reduction in water infiltration and retention [19]. On one hand, the limited infiltration increases flood risk, particularly in urban area with inadequate drainage systems, and constrains the groundwater recharge capacity, on the other, the reduced water retention impairs drought resilience. Furthermore, runoff from sealed surfaces often carries pollutants such as heavy metals, hydrocarbons, and microplastics into nearby soils and water bodies, posing additional contamination risks.

Soil sealing also leads to a decline in biodiversity across multiple ecological levels, by disrupting habitats for both belowground and aboveground organisms, including soil fauna (nematodes, arthropods, and earthworms), microorganisms (bacteria, fungi, and algae), plants, insects, other invertebrates, and vertebrates such as amphibians, reptiles, and small mammals [9]. Additionally, habitat fragmentation and the interruption of ecological corridors further contribute to aboveground biodiversity loss, resulting in species isolation and a reduction in ecosystem resilience [15].

The increase of sealed surfaces also exacerbates the urban heat island effect, particularly in densely built cities with limited vegetation, since impervious pavements absorb and retain more heat than soil [20], and loss of vegetation reduces evapotranspiration and shading, amplifying local warming. Finally, from a biogeochemical perspective, soil sealing interrupts the processes of nutrient cycling and organic matter decomposition, leading to a permanent loss of carbon storage capacity.

Together, these processes generate cumulative and synergistic impacts that extend beyond the sealed area, altering hydrological dynamics, local climatic conditions, and ecological integrity of surrounding environment.

Policy Frameworks and Mitigation Strategies

The European Commission has recognized soil sealing as a major environmental concern and, in 2012, published the *Guidelines on Best Practices for Limiting, Mitigating, or Compensating Soil Sealing* [14]. However, these guidelines are non-binding, and their implementation has varied widely across countries, resulting in a fragmented and inconsistent policy response.

In 2021, the European Commission launched the EU Soil Strategy for 2030, aiming to achieve healthy and resilient soils by 2050. The strategy identifies limiting land take and soil sealing as a priority and highlights the importance of circular land use. By revising the EU Soil Sealing Guidelines (2012), it aims to establish a common methodology to limit and mitigate these threats, while encouraging best practices based on the experiences of Member States with successful urban planning systems.

Coordinated policy action, consistent monitoring, and a shift in urban planning based on regeneration rather than on expansion, are essential to ensure that urban soils continue to support healthy and resilient urban environments [4]. While awaiting comprehensive guidance at the European level, some countries have already introduced national or regional targets to limit land take, implemented fiscal instruments to discourage development on agricultural land, and supported de-sealing initiatives to restore previously covered soils [15]. In Italy, policies on land take show initial progress but remain fragmented and constrained by the absence of a unified national regulatory framework [18].

DESEALING PRACTICES AND BENEFITS

In recent decades, de-sealing – also known as depaving – has been recognized as an effective measure to contrast the impacts of soil sealing and land take, and represent a valuable pathway to soil restoration and urban climate adaptation. The process involves the removal of the impermeable surfaces, and it is usually followed by other interventions such as the decompaction of the underlying materials, amendments addition, establishment of vegetation and other treatments aimed at promoting soil and ecosystem recovery [16,21,22]. Several desealing initiatives have been already implemented all over the world, following both top-down and bottom-up approaches. In top-down processes, depaving is integrated into larger urban planning and regeneration strategies, with projects that usually involve the replacement of specialized functions, as railway, airports and brownfields – disused industrial and artisanal sites – with new functions characterized by a mix of uses [Soil4life]. In contrast, in bottom-up models the actions are initiated and supported by residents, associations, or local stakeholders, and are mainly focused on the regeneration of over-paved and underutilized public spaces, such as squares, parking lots, residual spaces, and small urban voids [23]. These community-driven projects usually aim to the creation parks and community gardens to enhance both the quantity and the quality of public spaces [Soil4life]. Integrating these small interventions into a coherent green infrastructure network and municipal urban plan is essential to maximize ecological benefits, strengthen urban resilience and enhance urban biodiversity [22,24].

The desealing approach challenges decades of spatial planning based on urban expansion and car-centric design. One of the earliest and most influential desealing initiatives occurred in the early 2000s in North America, led by the nonprofit organization Depave, based in Portland, Oregon. Since 2008, Depave has collaborated with local communities to transform more than 34,000 square meters of underutilized pavement into gardens, rainwater infiltration zones, and public green spaces, demonstrating the social and environmental potential of desealing projects [25].

Across Europe, numerous cities have implemented large-scale desealing projects primarily focused on brownfield redevelopment, where former industrial, port, and railway areas have been transformed into multifunctional neighborhoods integrating residential, commercial, and recreational uses. Notable examples include Aalborg (Denmark) and Paris (France), where former railway yards have been converted into eco-districts designed to enhance climate-change adaptation. Similarly, former military area have been redeveloped into sustainable neighborhoods in Grenoble and Angers (France), while in Berlin (Germany), a disused railway yard and a former airport have been transformed into an urban park and a science and technology district, respectively [24]. Also Italian cities such as Milano, Torino and Trento have incorporate desealing as part of large brownfield sites regeneration [26].

A primary objective of these interventions is to restore soil permeability and mitigate hydrological risks by realizing rain gardens, retention basins, and other sustainable urban drainage systems (SUDS). Desealing interventions combined with such nature-based approaches have proven effective in reducing urban runoff and improving water quality [26–28]. Eco-districts exemplify integrated approaches, combining energy-efficient buildings, green infrastructure, and effective water management to improve urban microclimate, biodiversity, and water retention. These initiatives often integrate also blue-green corridors, linking urban design with ecological performance [24].

Recent studies have demonstrated that desealing and the implementation of green infrastructure contribute to local cooling effects and heatwaves mitigation [23,29,30]. Such climate-regulating benefits typically represents the second major objective of these interventions [22].

Beyond hydrological and microclimate benefits, soil desealing plays a crucial role in restoring biodiversity and enhancing soil ecological functions. By returning to a more natural state, desealed soils provide suitable conditions for the restoration of soil biological activity and for the establishment of native and pioneer vegetation. This, in turn, supports the development of diverse microbial and faunal communities which are essential for maintaining soil structure, fertility, and overall ecosystem multifunctionality [31].

Desealing and circularity: from demolition waste to constructed soils

Despite its potential benefits desealing faces considerable challenges. Critical aspects, such as the assessment of pre-existing soil conditions and the evaluation of post-intervention soil recovery and evolution, are often insufficiently addressed, limiting the effectiveness of restoration interventions [22]. Furthermore, common practices such as importing fertile topsoil can imply substantial economic and environmental costs, including CO_2 emissions and the loss of valuable agricultural land [14,22].

Additionally, desealing requires demolition activities and therefore it generates construction and demolition waste (CDW) – class 17 of the European Waste Catalogue (EWC) – an heterogeneous stream of materials, including concrete, bricks, asphalt, metals, wood, glass and plastics [32]. CDW is recognized as a priority waste stream under the Waste Framework Directive [33], and the EU Construction and Demolition Waste Management Protocol [34] provides guidelines and best practices for handling CDW. The protocol promotes pre-demolition audits, selective material separation – to safely manage and remove hazardous substances – and strategies to maximize material recovery, reuse and recycling. Effective CDW management is essential due to both its volume and potential as a secondary resource. Accounting for approximately 40% of Europe's total waste and projected to double by 2050 [35,36], CDW can be processed into valuable recycled aggregates (RAs). In 2018, over 70% of the mineral fraction was recovered across EU countries [36], but only a small fraction was reincorporated into new construction: 11% is used for backfilling, 18% landfilled, while the remaining is mostly down-cycled for applications like road bases [34,37].

Proper management of CDW generated during desealing, in accordance with circular economy principles, EU directives and protocols, is crucial to maximize environmental and economic efficiency. In recent years, some projects have explored the in situ reuse of CDW to minimize transport and disposal costs. For instance, in Dunkerque and Auberville (France), rock gardens were successfully developed by reusing asphalt blocks and demolition debris [24].

Concurrently, an increasing number of researches focused on the potential reuse of CDW and RAs as components of constructed or engineered soils. These soils, often referred to as constructed Technosols [38], are specifically designed to mimic natural soil functions while providing targeted, site-specific ecosystem services. Field and laboratory research has demonstrated that the incorporation of crushed concrete and milled asphalt, can improve soil structural properties and serve as effective growth substrates for various plant species. Coarse RAs, in particular, enhance soil macroporosity, thereby improving air and water flow, and can be used as structural materials to mitigate soil compaction in urban areas [39–43]. However, these studies also reveal significant knowledge gaps regarding the chemical behavior of such materials in soil, as well as their effects on percolation water and microbial activity [41,43,44], highlighting the need for careful assessment of their composition, quality, and contamination risks before application in soil.

In conclusion, effects of CDW and RAs remain largely unexplored due to the recent development of this research area, the limited number and duration of available studies. Moreover, differences in material composition, particle size distribution, and application rates may lead to variable outcomes and environmental responses.

RESEARCH GOALS AND THESIS OUTLINE

Many projects have highlighted the ecological and social benefits of desealing, recognizing it as a key strategy for restoring soil functions and enhancing urban resilience. At the same time, the in situ reuse of demolition waste and RAs for the creation of constructed soils offers promising opportunities within circular desealing practices. Nevertheless, significant knowledge gaps remain regarding the development and functioning of desealed soils, and environmental concerns – related to material quality, contamination risks, and long-term stability, arise from the use of demolition materials in soil restoration. This work aims to address some of these gaps and contribute to increase knowledge on the following topics: i) the physical, chemical and biological evolution of soil after desealing, and ii) the effects of different recycled materials on soil properties and functioning, including nutrient cycle and vegetation development.

This thesis is arranged into six chapters. This introduction (Chapter 1), which provides an overview of urban soils, soil sealing, desealing, and the use of CDW for constructed Technosols, is followed by a collection of four papers (one published and three in preparation).

Chapters 2 and 3 describe the outcomes of the desealing field experiment conducted in Milan between 2023 and 2024. This experiment aimed to investigate the effects of soil desealing on pedoclimatic conditions and early recovery processes under field conditions, to evaluate the potential and environmental suitability of concrete-based RAs as surface layers and growth substrates, and to assess the role of compost addition and vegetation establishment in driving soil recovery following desealing. In particular, Chapter 2 focuses on the evolution of pedoclimatic parameters (soil temperature, soil water content and oxygen concentration) by comparing sealed and desealed soils, and evaluates the environmental safety of RAs assessing the release of heavy metals in percolation water. Chapter 3 investigates not only the influence of RAs, but also the effects different soil restoration practices (compost addition and vegetation establishment) on the temporal evolution of soil properties, bacterial communities, and plant growth.

While the field experiment used coarse RAs (0-4 cm) derived from concrete based demolition waste at the experimental site, Chapter 4 presents an indoor-mesocosm experiment designed to investigate the effects, under controlled conditions, of the fine fraction (0-1 cm) of two different types of aggregates: recycled concrete aggregates (RCA) and reclaimed asphalt pavement (RAP). This experiment specifically aims to evaluate the effects of these recycled materials on biological endpoints (enzymatic activities, microbial basal respiration and phytotoxicity test) as well as on soil physicochemical properties.

Since the previous studies applied high amounts of aggregates in the soil (a pure surface RAs layer in the field experiment and 50% by volume in the indoor mesocosms), and considering the concerns regarding RCA phytotoxicity raised by the mesocosm study, Chapter 5 describes a novel rhyzobox experiment. This experiment was designed to investigate the effects of increasing concentrations of concrete-based RA in soil on plant development. As a preliminary study it focuses on the fine fraction of RA to determine appropriate soil-aggregates proportion for future research.

Finally, Chapter 6 provides a synthesis of the overall outcomes is provided and discusses future perspectives.

Bibliography

1. Morel, J.L.; Chenu, C.; Lorenz, K. Ecosystem Services Provided by Soils of Urban, Industrial, Traffic, Mining, and Military Areas (SUITMAs). *J Soils Sediments* 2015, *15*, 1659–1666, doi:10.1007/s11368-014-0926-0.
2. *Urban Soils*; Rate, A.W., Ed.; Springer International Publishing: Cham, 2022; ISBN 978-3-030-87315-8.
3. Pereira, P.; Bogunovic, I.; Muñoz-Rojas, M.; Brevik, E.C. Soil Ecosystem Services, Sustainability, Valuation and Management. *Curr Opin Environ Sci Health* 2018, *5*, 7–13, doi:10.1016/j.coesh.2017.12.003.
4. Food and Agriculture Organization of the United Nations *Status of the World's Soil Resources (SWSR) – Main Report*; 2015;
5. O'Riordan, R.; Davies, J.; Stevens, C.; Quinton, J.N. The Effects of Sealing on Urban Soil Carbon and Nutrients. *SOIL* 2021, *7*, 661–675, doi:10.5194/soil-7-661-2021.
6. Ungaro, F.; Maienza, A.; Ugolini, F.; Lanini, G.M.; Baronti, S.; Calzolari, C. Assessment of Joint Soil Ecosystem Services Supply in Urban Green Spaces: A Case Study in Northern Italy. *Urban For Urban Green* 2022, *67*, 127455, doi:10.1016/j.ufug.2021.127455.
7. IUSS Working Group WRB. 2022. World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. 4th Edition. International Union of Soil Sciences (IUSS), Vienna, Austria.
8. O'Riordan, R.; Davies, J.; Stevens, C.; Quinton, J.N.; Boyko, C. The Ecosystem Services of Urban Soils: A Review. *Geoderma* 2021, *395*, 115076, doi:10.1016/J.GEODERMA.2021.115076.
9. Filazzola, A.; Shrestha, N.; MacIvor, J.S. The Contribution of Constructed Green Infrastructure to Urban Biodiversity: A Synthesis and Meta-analysis. *Journal of Applied Ecology* 2019, *56*, 2131–2143, doi:10.1111/1365-2664.13475.
10. European Commission; Directorate-General for Research and Innovation; Veerman, C.; Pinto Correia, T.; Bastioli, C.; Biro, B.; Bouma, J.; Cienciala, E.; Emmett, B.; Frison, E.A.; et al. *Caring for Soil Is Caring for Life – Ensure 75% of Soils Are Healthy by 2030 for Food, People, Nature and Climate – Report of the Mission Board for Soil Health and Food*; Publications Office, 2020; ISBN 9789276216032.
11. Stolte, J.; Tesfai, M.; Øygarden, L.; Kværnø, S.; Keizer, J.; Verheijen, F.; Panagos, P.; Ballabio, C.; Hessel, R. *Soil Threats in Europe – Status, Methods, Drivers and Effects on Ecosystem Services*; Publications Office of the European Union, 2015;

12. Schillaci, C.; Jones, A.; Vieira, D.; Munafò, M.; Montanarella, L. Evaluation of the United Nations Sustainable Development Goal 15.3.1 Indicator of Land Degradation in the European Union. *Land Degrad Dev* 2023, *34*, 250–268, doi:10.1002/ldr.4457.
13. Binner, H.; Wojda, P.; Yunta, F.; Breure, T.; Schievano, A.; Massaro, E.; Jones, A.; Newell, J.; Paradelo, R.; Popescu Boajă, I.; et al. A Systematic Review and Characterization of the Major and Most Studied Urban Soil Threats in the European Union. *Water Air Soil Pollut* 2024, *235*, 494, doi:10.1007/s11270-024-07288-x.
14. European Commission and Directorate-General for Environment *Guidelines on Best Practice to Limit, Mitigate or Compensate Soil Sealing*; Publications Office, 2012;
15. The State of Soils in Europe - Publications Office of the EU Available online: https://op.europa.eu/en/publication-detail/-/publication/1f96158b-901f-11ef-a130-01aa75ed71a1/language-en (accessed on 30 October 2025).
16. Tobias, S.; Conen, F.; Duss, A.; Wenzel, L.M.; Buser, C.; Alewell, C. Soil Sealing and Unsealing: State of the Art and Examples. *Land Degrad Dev* 2018, *29*, 2015–2024, doi:10.1002/ldr.2919.
17. Imperviousness and Imperviousness Change in Europe | Indicators | European Environment Agency (EEA) Available online: https://www.eea.europa.eu/en/analysis/indicators/imperviousness-and-imperviousness-change-in-europe?utm_source=chatgpt.com (accessed on 30 October 2025).
18. ISPRA – Istituto Superiore per la Protezione e la Ricerca Ambientale *Consumo Di Suolo, Dinamiche Territoriali e Servizi Ecosistemici. Edizione 2025*; 2025;
19. Pistocchi, A.; Calzolari, C.; Malucelli, F.; Ungaro, F. Soil Sealing and Flood Risks in the Plains of Emilia-Romagna, Italy. *J Hydrol Reg Stud* 2015, *4*, 398–409, doi:10.1016/J.EJRH.2015.06.021.
20. Nwakaire, C.M.; Onn, C.C.; Yap, S.P.; Yuen, C.W.; Onodagu, P.D. Urban Heat Island Studies with Emphasis on Urban Pavements: A Review. *Sustain Cities Soc* 2020, *63*, 102476, doi:10.1016/j.scs.2020.102476.
21. Maienza, A.; Ungaro, F.; Baronti, S.; Colzi, I.; Giagnoni, L.; Gonnelli, C.; Renella, G.; Ugolini, F.; Calzolari, C. Biological Restoration of Urban Soils after De-Sealing Interventions. *Agriculture (Switzerland)* 2021, *11*, 1–12, doi:10.3390/agriculture11030190.
22. Vieillard, C.; Vidal-Beaudet, L.; Dagois, R.; Lothode, M.; Vadepied, F.; Gontier, M.; Schwartz, C.; Ouvrard, S. Impacts of Soil De-Sealing Practices on Urban Land-Uses, Soil Functions and Ecosystem Services in French Cities. *Geoderma Regional* 2024, *38*, e00854, doi:10.1016/j.geodrs.2024.e00854.
23. Caselli, B.; Ceci, M.; De Noia, I.; Garda, E.; Zazzi, M. Towards the Integration of Soil Desealing in the Urban Areas' Transformation Processes. In; 2024; pp. 286–298.
24. *AZIONE A1.3 - Sintesi Di Norme, Linee Guida, Buone Pratiche, Casi Studio in Materia Di Limitazione Di Consumo Di Suolo e Resilienza Urbana al Cambiamento Climatico - SOS4LIFE - Save Our Soil for Life*; 2017;
25. Depave *Impact Report 2022-2023*; Portland, OR, USA, 2023;
26. Garda, E. Greening The Void: Actions For The De-Sealing And Renaturalisation Of Soils In Brownfield Regeneration Processes. 2024, *36*, 366–366.
27. Adobati, F.; Garda, E. Soil Releasing as Key to Rethink Water Spaces in Urban Planning. *City, Territory and Architecture* 2020, *7*, 9, doi:10.1186/s40410-020-00117-8.
28. Scheiber, L.; Teixidó, M.; Criollo, R.; Labad, F.; Vázquez-Suñé, E.; Izquierdo, M.; Chesa Marro, M.J.; de Castro, D. ASSET Project: Assessing Sustainable Urban Drainage System (SUDS) Efficiency to Reduce Urban Runoff Water Contamination. *Advances in Geosciences* 2022, *59*, 37–44, doi:10.5194/adgeo-59-37-2022.
29. Aimar, F. Limiting Soil Sealing and Depaving: Local Actions for Regenerating Public Spaces to Build Green Infrastructures. In; 2023; pp. 127–137.
30. Falco, E.; Garda, E.; Zardo, L.; Cortinovis, C. Learning from Two Early Brownfield Redevelopment Projects in Italy: Soil Desealing, Cooling Effects, and Implementation of Nature-Based Solutions Through Traditional Planning Tools. *Land (Basel)* 2024, *13*, 1700, doi:10.3390/land13101700.
31. Renella, G. Evolution of Physico-Chemical Properties, Microbial Biomass and Microbial Activity of an Urban Soil after de-Sealing. *Agriculture (Switzerland)* 2020, *10*, 1–11, doi:10.3390/agriculture10120596.
32. COMMISSION DECISION of 18 December 2014 Amending Decision 2000/532/EC on the List of Waste Pursuant to Directive 2008/98/EC of the European Parliament and of the Council . *Official Journal of the European Union* 2014.
33. European Parliament & Council of the European Union Directive 2008/98/EC on Waste and Repealing Certain Directives. *Official Journal of the European Union, L 312, pp. 3-30* 2008.

34. European Commission; Directorate-General for Internal Market, I.E. and Sme.; Oberender, A.; Fruergaard Astrup, T.; Frydkjær Witte, S.; Camboni, M.; Chiabrando, F.; Hayleck, M.; Akelytė, R. *EU Construction & Demolition Waste Management Protocol Including Guidelines for Pre-Demolition and Pre-Renovation Audits of Construction Works – Updated Edition 2024*; Publications Office of the European Union, 2024;
35. Eurostat Waste Statistics - Statistics Explained Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Waste_statistics (accessed on 29 October 2025).
36. Damgaard, A.; Lodato, C.; Butera, S.; Fruergaard, T.F.; Kamps, M.; Corbin, L.; Tonini, D.; Astrup, T.F. Background Data Collection and Life Cycle Assessment for Construction and Demolition Waste (CDW) Management. *JRC130992* 2022, doi:10.2760/772724.
37. Giorgi, S. *Progettare La Circolarità*; 2024; ISBN 9788835129110.
38. Fabbri, D.; Pizzol, R.; Calza, P.; Malandrino, M.; Gaggero, E.; Padoan, E.; Ajmone-Marsan, F. Constructed Technosols: A Strategy toward a Circular Economy. *Applied Sciences (Switzerland)* 2021, *11*, doi:10.3390/app11083432.
39. Coull, M.; Butler, B.; Hough, R.; Beesley, L. A Geochemical and Agronomic Evaluation of Technosols Made from Construction and Demolition Fines Mixed with Green Waste Compost. *Agronomy* 2021, *11*, doi:10.3390/agronomy11040649.
40. Rompato, B.; Rossi, R.; Giagnoni, L.; Mastrolonardo, G.; Argenti, G.; Staglianò, N.; Nuñez, R.P.; Certini, G. Removed Asphalt Can Be Used as a Component of Constructed Soils for Urban Greenery. *J Soils Sediments* 2025, doi:10.1007/s11368-024-03952-1.
41. Mastrolando, G.; Giagnoni, L.; Maienza, A.; Fantoni, G.; Rompato, B.; Argenti, G.; Selvi, F.; Fiore, B.; Certini, G. Use of Waste Materials as Feasible Strategy for Urban De-Sealed Soil Reclamation: Evidence from a Restoration Case Study in Prato, Italy. In Proceedings of the 13th International Symposium of the International Union of Soil Sciences on Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA 13); 2025; pp. 158–159.
42. Yilmaz, D.; Cannavo, P.; Séré, G.; Vidal-Beaudet, L.; Legret, M.; Damas, O.; Peyneau, P.-E. Physical Properties of Structural Soils Containing Waste Materials to Achieve Urban Greening. *J Soils Sediments* 2018, *18*, 442–455, doi:10.1007/s11368-016-1524-0.
43. Mikajlo, I.; Pando, A.; Robain, H.; Lerch, T.Z. Reusing Asphalt Millings with Excavated Materials and Compost to Construct Technosols: Effects on Soil Properties and Plant Growth. *J Soils Sediments* 2024, doi:10.1007/s11368-024-03942-3.
44. Herbreteaua, A.; Schwartzb, C.; Beguiristaina, T.; Bojicc, C.; Cossu-Leguillec, C.; Durandb, A.; Leglizeb, P.; Lorgeouxd, C.; Faurea, P. Reactivity of Road Asphalt Buried in Soils after De-Sealing Operations. In Proceedings of the 13th International Symposium of the International Union of Soil Sciences on Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA 13); 2025; pp. 205–206.

CHAPTER 2

Soil de-sealing and recycled aggregates application: one year of monitoring

Gaia Mascetti, Roberto Comolli, Francesca Pittino, Isabella Gandolfi and Chiara Ferré

DISAT-Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano Bicocca, Milano, Italia

Published

Mascetti, G., Comolli, R., Pittino, F., Gandolfi, I., & Ferré, C. (2025). Soil De-Sealing and Recycled Aggregates Application: One Year of Monitoring. Soil Systems, 9(4), 128. https://doi.org/10.3390/soilsystems9040128

Abstract

De-sealing, or depaving, is increasingly adopted to restore soil permeability and support green infrastructure, yet its potential to recover soil functions remains insufficiently understood. This study reports one year of soil monitoring following the de-sealing of a brownfield site in Milan (Italy). It compares the evolution of pedoclimatic parameters in sealed and de-sealed soils and assesses the suitability of recycled aggregates (RAs) from demolition waste as a soil constituent material. Buried sensors continuously recorded pedoclimatic parameters, temperature, water content, and oxygen concentration, while periodic sampling was carried out to analyze soil chemical properties, bacterial community composition, and the quality of percolation water (heavy metal content). De-sealing immediately improved pedoclimatic conditions, enhancing soil aeration, water regulation, and heat exchange capacity. No significant variations were detected in soil chemical properties, apart from pH fluctuations linked to the leaching of alkaline ions from concrete-based RAs. The presence of RAs caused no adverse effects on either soil or percolation water. Bacterial community composition was strongly associated with soil organic carbon, C:N ratio, and soil water content, without showing clear temporal trends. Overall, the study demonstrates that de-sealing rapidly triggers soil functional recovery and that, when properly characterized for composition and contamination risk, RAs pose no evident threat to the surrounding environment.

INTRODUCTION

Soil sealing is the permanent covering of soil with impermeable artificial materials such as asphalt, concrete, or paving stones. Driven by urbanization, it causes rapid land consumption, often at the expense of arable land [1], and is a major driver of soil degradation in Europe [2,3]. It disrupts interactions among the soil, atmosphere, and hydrosphere, disturbing biogeochemical and hydrological cycles and impairing soil functions and ecosystem services. The most evident consequences include habitat loss and fragmentation, reduced soil productivity and infiltration capacity, and an intensification of the urban heat island effect [3].

In recent decades, de-sealing (or depaving) has emerged as an important strategy to mitigate and offset the impacts of soil sealing and to support European targets for reducing land take and achieving the Sustainable Development Goal of making cities and human settlements inclusive, safe, resilient, and sustainable [4]. The process entails removing the impermeable surface layer, followed by the decompaction of underlying materials and their replacement with a permeable pavement or the reconstruction of the soil profile [5–7].

Numerous de-sealing projects have already been implemented worldwide, ranging from large-scale urban regeneration initiatives, such as converting railways, airports, or brownfields into mixed-use developments [8], to small-scale actions led by communities, associations, and local stakeholders [9]. These smaller efforts often target over-paved public areas (e.g., squares, car parks, and residual urban spaces), fostering the creation of green areas, parks, and community gardens. Integrating such interventions into a coherent, well-planned green infrastructure network is essential to maximize ecological benefits and can significantly enhance urban biodiversity [7,8]. In Italy, several cities have recently adopted depaving strategies; however, according to ISTAT data, the increase per capita urban green space has been modest, rising from 31.9 m^2 in 2011 to 33.3 m^2 in 2023 [10], suggesting that current efforts remain insufficient to offset ongoing soil sealing and that broader de-sealing interventions are urgently needed.

De-sealing is currently applied primarily to restore soil permeability and implement green infrastructure [11], thereby enhancing urban resilience to flooding and heatwaves [9]. A survey of French cities [7] confirmed that the main goals of de-sealing interventions are sustainable rainwater management and heat mitigation. De-sealing also plays a key role in restoring biodiversity across multiple ecological levels by creating favorable conditions for the establishment of plants, animals (such as nematodes, arthropods, and earthworms), prokaryotes, and fungi. Among these groups, all essential for improving soil properties and functions [12], prokaryotic communities are the main biological drivers of biogeochemical cycles. They are involved in the decomposition of organic matter, including organic carbon mineralization, nitrification, denitrification, and phosphorus solubilization. By converting chemical compounds into nutrients available to other organisms, these microorganisms enhance soil fertility and functionality [13].

Despite its advantages, de-sealing must be implemented with technical rigor, involving site-specific assessments to identify existing contamination, detect possible pollutant migration pathways, and evaluate the feasibility of the intervention. Information on soil and material properties is also crucial to ensure the effectiveness and long-term sustainability of de-sealing projects [7,14], depending on the intended post-intervention use. However, there are still no specific guidelines or tools for optimizing de-sealing and soil restoration practices [7]. According to Caselli et al. [9], de-sealing has appeared in the scientific literature since the 1990s. Yet research has mainly focused on its integration into urban planning, with limited attention to its direct role in soil restoration and multifunctionality. Its significance as an independent soil management strategy has only recently been recognized [9]. Recent studies have demonstrated the benefits of de-sealing for urban and peri-urban environments [15] and have shown that de-sealed soils can regain their functions and, consequently, provide associated ecosystem services even without direct intervention, particularly when colonized by spontaneous vegetation [12]. Nevertheless, the recovery of physical, chemical, and biological fertility in newly de-sealed soils can be enhanced through decompaction and targeted treatments [6]. These typically involve applying exogenous topsoil, sometimes enriched with organic or mineral amendments, to improve soil properties and facilitate plant establishment [3,6,7]. De-sealed soils are often subsequently vegetated by seeding or planting appropriate species selected according to soil characteristics, climate, and intended land use [7,11]. A well-adapted plant cover can improve soil quality, support biodiversity, strengthen urban resilience to climate change, and reduce maintenance requirements [7].

However, the extraction, transport, and application of imported topsoil, which may derive either from agricultural soils or, more frequently, from urban soils excavated during construction activities (e.g. building foundations), entail significant economic and environmental costs, including off-site soil loss and increased CO_2 emissions [3,7]. In line with circular economy principles, a more sustainable alternative is to reuse locally derived materials, following a detailed assessment of their composition, quality, and contamination risk [7]. According to Villeiard et al. [7], recent studies have explored the reuse of various organic and mineral waste materials, including construction and demolition waste (C&DW). The inert, non-hazardous fraction of C&DW (CER 17 09 04) [16] can be processed into recycled aggregates (RAs) through on-site crushing and screening [17] and reused according to their properties and composition. For instance, concrete waste from underground structures can yield high-quality RAs suitable for structural applications, while mixed C&DW can produce aggregates for non-structural uses such as backfilling [16]. The on-site reuse of RAs exemplifies applied circular economy principles, reducing both the economic and environmental costs associated with transporting and disposing of C&DW, the largest waste stream in Europe, accounting for approximately 40% of total waste [18]. In recent years, several de-sealing projects, such as those in Dunkerque and Auberville (France), have reused asphalt blocks and demolition debris in situ as substrate material for urban green installations [8]. Scientific trials have indicated that RAs can serve as cost-effective and promising soil constituent materials [1,19]. However, the environmental safety of recycled aggregates strongly depends on their composition, particle size, degradation rate, and potential contamination risks, including alkalinity or heavy metal release. Moreover, since RAs are largely inorganic material, their use in soil reconstruction may require the addition of organic amendments.

Overall, de-sealing represents a valuable and increasingly adopted strategy for mitigating the impacts of soil sealing and supporting urban regeneration. However, as a relatively new topic in soil science, its potential to restore soil multifunctionality – physical, chemical, and biological functions supporting soil health – remains poorly understood. Previous studies have primarily investigated individual soil functions, such as enhanced permeability [15], and selected aspects of chemical and biological fertility [6,12]. To date, some research has examined the effects of de-sealing and revegetation on the urban microclimate [20], but no studies have assessed pedoclimatic parameters in sealed and de-sealed soils or their relationships with soil biological and physical processes. Moreover, despite growing interest, few studies have evaluated the effects of recycled aggregates on soil properties and functions [1,19], and none under real field conditions. Given the high heterogeneity of RAs, arising from the variable composition and origin of their parent C&DW materials, further research is essential to assess their performance and suitability as soil reconstruction materials.

This study addresses these gaps through a field monitoring experiment that integrates pedoclimatic and microbiological analyses in de-sealed urban soils amended with RAs. Specifically, it presents the results of one year of monitoring following a de-sealing intervention aimed at promoting soil functional recovery and assessing the use of recycled aggregates as a soil constituent material and growth substrate for vegetation. The study's objectives were to: (i) compare the evolution of pedoclimatic parameters (temperature, water content, and oxygen concentration) in sealed and de-sealed soils; (ii) monitor changes in soil properties and bacterial communities in de-sealed soils; and (iii) evaluate these out-comes in relation to the addition of RAs as a constituent material of the soil.

MATERIAL AND METHODS

Study Area and Experimental Design

The study area lies within a broader de-sealing experimental site, an urban brownfield in south-eastern Milan, Italy (45°25'27.0" N; 9°14'0.9" E), managed by the Urban Forestry Centre (CFU), the operational branch of the national association "Italia Nostra". In 2022, a preliminary soil investigation was carried out on the soils of the study area which, being sealed, were classified as Ekranic Technosols [21]. The investigation confirmed the absence of soil contamination beneath the sealing layer and the feasibility of the de-sealing project.

De-sealing was carried out in October 2023, following project approval, acquisition of equipment, and coordination with the Urban Forestry Centre. After removal of the concrete cover, a pedological survey was conducted by opening and describing four soil profiles (Figure S1; Table S1). The profiles revealed weakly developed, base-saturated soils with layers formed from material moved by intentional human activity, classified as Eutric Transportic Regosols [21]. The bacterial communities in these profiles were analyzed using the same methods described in Section 2.4 (Figure S2).

The de-sealing and soil characterization were completed on 17 October 2023 (time zero, t_0), marking the start of discrete monitoring of soil properties. The following week, a soil monitoring system comprising 16 buried sensors was installed to continuously record soil temperature (T), soil water content (SWC), and soil air oxygen concentration (O_2) from 26 October 2023 to 29 October 2024.

Sensors were placed approximately 30 cm below both the newly exposed soil surface in four experimental plots (D1–D4) and beneath the remaining concrete pavement at two sites (S1 and S2; Figure 1a), to monitor pedoclimatic conditions under three scenarios: de-sealed soil, centrally sealed soil (S1), and sealed soil at the pavement edge (S2). The latter represented a partially sealed condition caused by edge effects, typical of road and sidewalk margins. Owing to the malfunction of the oxygen sensor at the central sealed position (S1), a theoretical oxygen concentration of 1 % v/v was assumed for well-sealed soil, representing anaerobic conditions in line with literature values for oxygen depletion under impermeable layers [22–24].

In the de-sealed plots, additional temperature and soil water content sensors were installed at a 10 cm depth (Figure 1b) to assess vertical gradients in temperature and moisture. All sensors (Apogee SO-110 oxygen sensors and Meter Teros-11 soil water content and temperature sensors) were connected to a data acquisition system comprising a Campbell Scientific AM16/32B multiplexer and a Campbell Scientific CR300 data logger, powered by a 12 V–60 Ah gel buffer battery. The system was programmed for hourly data collection and housed in a waterproof container. On-site meteorological parameters (air temperature and precipitation) were also recorded using a Rain Gauge RG3-M equipped with a HOBO Data Logger UA-003, to provide contextual environmental data.

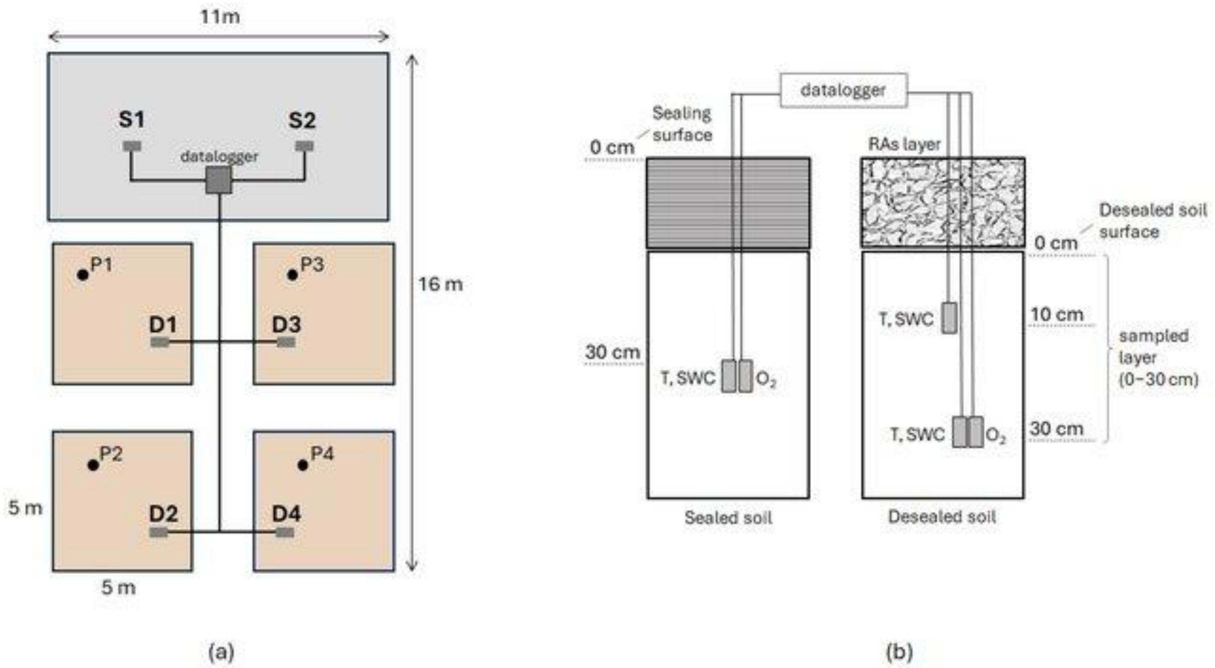


Figure 1. Experimental design and soil monitoring system. **(a)** Spatial distribution of sensors in de-sealed (D1, D2, D3 and D4) and sealed soil (S1 and S2). The figure also shows the location of the soil profiles (P1, P2, P3 and P4) in the experimental plots. **(b)** Graphical representation of the sensors buried under the pavement and RA layer in sealed and de-sealed soil respectively, for the monitoring of temperature (T), soil water content (SWC) and oxygen (O2) in soil air.

Specially designed drainage lysimeters, cylindrical soil-filled containers open at the top to allow water infiltration and equipped with a collection system at the base, were also installed in the de-sealed soil to collect percolated water and detect any contaminants potentially released by the use of RAs (Figure S3). The recycled aggregates, obtained from crushed concrete pavement, were evenly spread over the de-sealed soil to form a 20 cm layer serving as a soil-like substrate for vegetation growth. Five lysimeters were installed: four within the experimental plots (one per plot) and one in a control area adjacent to the site, where the de-sealed soil was left uncovered by the RA layer.

According to national and European regulations [25], demolition waste from the concrete pavements and, in part, from abandoned buildings was characterized and classified as non-hazardous (CER 17 09 04) [16]. This material was then crushed and sieved to a maximum particle size of 4 cm to produce recycled aggregates (RAs), composed mainly of concrete, sand, bricks, and stones, with observed traces of metal, glass, rubber, and plastic. Before field application, the producer carried out a leaching test on the RAs.

On 5 April 2024, green waste compost was incorporated into two plots (D3 and D4), and all plots were subsequently sown: two with a monoculture of Sorghum bicolor (D1 and D3) and two with a mixture of Lolium and Trifolium species (D2 and D4). The two vegetation types were selected to represent contrasting uses and functional traits; their implications are discussed in detail in Chapter 3.

Soil Sampling and Analyses

Changes in the properties of the de-sealed soil were monitored through periodic sampling of the 30 cm soil layer immediately beneath the 20 cm RA layer (Figure 1b). From October 2023 to January 2024, soil samples were collected monthly using a composite sampling approach. After removing the RAs with a shovel, three samples were taken from each plot using a gouge auger and then combined. Subsequently, sampling was performed every three months, in April, July, and October 2024, for a total of seven sampling events. These later samples were not collected using the composite method, as it can damage vegetation. Instead, each plot was divided into two halves: one for monitoring vegetation growth and the other subdivided into three smaller subplots (approximately 1.5 m × 2 m each). One soil sample was collected from each subplot at every sampling time.

Samples were air-dried at room temperature and sieved (2 mm mesh) for standard laboratory analyses: pH (measured potentiometrically in a 1:2.5 soil-to-water ratio), total carbonates (Dietrich-Frühling calcimeter), soil organic carbon (SOC), and total nitrogen (Flash EA 1112 NC-Soil elemental analyzer, Thermo Fisher Scientific CN, Pittsburgh, USA.

Leaching Test and Percolation Water

The potential release of pollutants from the RAs was evaluated before their application in the field. The leaching test, conducted by the RA supplier and detailed in the Supplementary Materials (Table S2), showed that the RA eluate had a slightly alkaline pH (8.7) and low concentrations of major ions (nitrates 34.6 mg/L, sulphates 244 mg/L, chlorides 6.3 mg/L), all below the regulatory limits. Likewise, concentrations of potentially toxic metals, including Cu (0.006 mg/L), Zn (<0.001 mg/L), Ni (2.61 μg/L), Pb (<10 μg/L), and Cd (<1 μg/L), were well below reference thresholds. These results confirm that the RAs were safe for use in soil reconstruction under the conditions of this study.

Percolation water was subsequently collected from the lysimeters every one to two months, depending on rainfall frequency and intensity, using a vacuum pumping system. The total concentrations of heavy metals (Pb, Cu, Ni, Cr, Zn) were determined by atomic absorption spectroscopy (AAnalyst 200 Atomic Absorption Spectrometer, PerkinElmer Inc.). Finally, metal concentrations were normalized to the volume of percolation water collected from each lysimeter.

Bacterial Community Characterization

Soil sampling for the analysis of the bacterial communities was carried out as described above for the soil properties, using nitrile gloves and cleaning all the equipment with ethanol prior to each sample sampling. Within 24 hours, approximately 5 g of each sample were stored at -20°C until further processing. For the characterization of the bacterial community, total DNA was extracted from 0.5 g of each soil sample using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Solon, OH, United States), according to manufacturer's instructions. Then, the V5–V6 hypervariable regions of the 16S rRNA gene were amplified by PCR using 783F and 1046R primers, and the resulting amplicons were sequenced by MiSeq Illumina with a 2 x 300 bp paired end protocol (Illumina, Inc., San Diego, CA, USA), as described in Nava et al. [26]. Sequence processing and Amplicon Sequence Variants (ASVs) inferring and classification were carried out as described in Gandolfi et al. [27].

Data Processing and Analysis

All statistical analyses and data processing were performed using RStudio software, version 4.4.2.

After processing the raw sensor data as described in Appendix A, a comprehensive pedo-climatic dataset was compiled by integrating daily data from 26 October 2023 to 29 October 2024. Missing values were estimated by linear interpolation, accounting for air temperature variations to ensure full temporal coverage (n = 370 observations). Summary statistics (mean, standard deviation, minimum, and maximum) were calculated for each variable and grouped by meteorological season, soil cover condition, experimental treatment, and soil depth to enable comparative analyses. Seasonal variations were assessed according to meteorological seasons (Winter: December–February; Spring: March–May; Summer: June–August; Autumn: September–November).

The effects of de-sealing, compost addition (CoA), and vegetation type (VgT) were analyzed using mean values: D1–D2–D3–D4 (de-sealed plots), D3–D4 (with compost), D1–D2 (without compost), D1–D3 (Sorghum), and D2–D4 (mixed grass). The effects of CoA and VgT on de-sealed soil parameters were evaluated over the period from 5 April 2024 to 29 October 2024 (n = 203 observations). As all environmental variables remained non-normally distributed after transformation (Shapiro–Wilk $p < 0.001$), Cliff's delta was applied to obtain robust, non-parametric estimates of effect size. Cliff's delta represents the probability that a random observation from one group exceeds that from another. To account for temporal dependencies, 95% confidence intervals were computed through bootstrap resampling (1000 iterations), preserving the temporal structure of the data while providing reliable uncertainty estimates.

Temporal changes in soil properties were analyzed using linear mixed models, with experimental plot included as a random factor (lme function). Spatial independence of residuals was assessed using Moran's I test based on a nearest-neighbor weight matrix. Model validity was checked with the Shapiro–Wilk test, and model selection was based on the Akaike Information Criterion (AIC). Accordingly, pH, $CaCO_3$, and C:N ratio were transformed using logarithmic, Yeo–Johnson, and Box–Cox transformations, respectively. Significant temporal differences between sampling dates (categorical variable) were evaluated through post hoc Tukey's tests on estimated marginal means (EMMs). For graphical representation, results were back-transformed to the original scale using the corresponding inverse functions and displayed as EMM plots.

The temporal dynamics of the soil bacterial community structure were examined at the order level, focusing on the ten most abundant taxa. Principal Component Analysis (PCA) was used to explore relationships among soil properties, pedoclimatic factors, and these ten dominant bacterial orders (prcomp function). For pedoclimatic variables, mean values from the two days preceding each sampling event were used. Linear mixed models with plot as a random effect (lme function) were then applied to assess the influence of environmental factors on bacterial orders. Among the pedological and climatic variables, significant predictors were first identified through stepwise model selection (stepAIC function) for each abundant order. Model assumptions were verified using Moran's I, the Shapiro–Wilk test, and diagnostic plots; when required, variables were transformed to satisfy normality and homoscedasticity of residuals. Model performance and accuracy were evaluated using marginal and conditional R^2 values and the Akaike Information Criterion.

RESULTS

Pedoclimatic Factors

Soil Temperature

Temperature differences were detected between sealed and de-sealed soils at a depth of 30 cm (Figure 2). Overall, the de-sealed plots maintained cooler soil temperatures than both sealed positions, with an annual mean of 15.9°C (Table 1). Seasonal analysis showed a strong cooling effect during summer (−7.2/−5.9°C compared to S1 and S2, respectively; Cliff's $|\delta| > 0.474$). Medium to small effects were observed in spring, while in winter temperatures remained relatively uniform across cover conditions, with occasional thermal reversals (Figure 2; Table 2). In general, de-sealing increased daily temperature fluctuations, especially during extreme weather events, yet reduced seasonal thermal extremes compared to sealed soils, producing lower summer and higher winter temperatures. Temperature differences between the sealed positions were negligible in autumn and winter, and small in spring and summer, with S2 consistently cooler than S1, indicating a temperature gradient across the sealing conditions.

Temperature profiles of the de-sealed soil at 10 and 30 cm depths followed similar overall trends (Figure 2), though diurnal and seasonal variability was more pronounced at 10 cm, reflecting the greater sensitivity of surface layers to meteorological conditions. Analysis of treatment effects in the de-sealed plots showed that compost addition and vegetation type had no significant influence on soil temperature (Cliff's $|\delta| < 0.147$; Table 3).

Soil water content

The effects of de-sealing on soil moisture differed clearly among the soil cover conditions. The de-sealed soil showed the highest SWC, with an annual mean of 25.7%, exceeding that of well-sealed soil by 5.2% and that of partially sealed soil by 3.9% (Table 1). The largest contrasts were observed between S1 and S2 (Cliff's $|\delta| > 0.474$). This general pattern (S2 > D > S1) persisted throughout the monitoring period with a large effect size, although seasonal dynamics varied considerably among cover conditions, largely due to differing moisture responses to rainfall events (Figure 2).

De-sealed soils were strongly influenced by meteorological drivers, showing the lowest SWC values in summer (Table 1; Figure 2) under drought and high-temperature conditions. Partially sealed soils (S2) responded most strongly to rainfall, reaching the highest water content values after heavy precipitation events (>15 mm), with moisture levels remaining elevated longer than in de-sealed plots (Figure 2). However, S2 was less affected by extreme summer drought. In contrast, the well-sealed soil (S1) exhibited a gradual, steady decline in water content that was largely independent of meteorological conditions.

In the de-sealed plots, vertical profiles showed similar SWC patterns at 10 cm and 30 cm depths, with greater fluctuations in the surface layer (Figure 2). Analysis of compost addition (CoA) and vegetation type (VgT) treatments indicated negligible effects on de-sealed soil moisture (Cliff's $|\delta| < 0.147$; Table 3).

Oxygen content in soil air

A marked increase in oxygen availability was recorded in the de-sealed plots compared with the sealed soils. Relative to S1, which, according to the literature, has a theoretical oxygen concentration of 1% v/v [22–24], de-sealing resulted in a clear shift from anaerobic to aerobic conditions (absolute difference +14.1%; Cliff's $\delta = 1$). A large effect was also detected relative to S2 (absolute difference +2.5%; Cliff's $|\delta| > 0.474$), although S2 was already under aerobic conditions (Table 2). A similarly large difference was found between S1 and S2 (Cliff's $\delta = 1$). The only deviation from this general pattern (D > S2 > S1) occurred at the onset of summer, when O_2 concentration in the de-sealed soil dropped markedly, falling below that of S2 (Figure 2; Table 1).

Treatment analysis showed that vegetation type had a strong effect on soil oxygen concentration (Cliff's $\delta = -0.824$), with mixed grass plots maintaining higher O_2 levels than sorghum monoculture plots (Table 3). In contrast, compost application had no significant effect.

Table 1. Seasonal and annual average values (mean ± sd) of pedoclimate parameters divided by soil cover groups (D= de-sealed; S1 = well sealed; S2 = partially sealed). For the de-sealed soil the mean values were calculated as the average of the experimental plots at 10 (D_{10}) and 30 cm (D_{30}).

Parameter	Period	D_{10}	D_{30}	S1	S2
T (°C)	Winter	6.6 ± 1.7	7.3 ± 1.3	6.9 ± 2	6.6 ± 2
	Spring	15.2 ± 3.6	14.8 ± 3.3	17.5 ± 4.3	16.5 ± 3.9
	Summer	25.1 ± 1.8	24.4 ± 1.8	31.6 ± 3.4	30.4 ± 3.5
	Autumn	16 ± 5.6	16.9 ± 5.2	18.6 ± 6.2	17.8 ± 6
	Annual	15.7 ± 7.4	15.9 ± 6.9	18.7 ± 9.7	17.8 ± 9.4
SWC (%)	Winter	27.1 ± 1.3	26.8 ± 1.4	22.4 ± 2.1	28.4 ± 4.9
	Spring	28.2 ± 1.7	28.2 ± 1.7	19.3 ± 0.4	31.9 ± 1.8
	Summer	20.8 ± 4.7	21.5 ± 4.7	18.3 ± 0.2	27.8 ± 1.6
	Autumn	25.8 ± 2.8	26.4 ± 2.9	21.8 ± 5.2	30.1 ± 4.4
	Annual	25.5 ± 4	25.7 ± 3.9	20.4 ± 3.3	29.6 ± 3.9

	Winter	-	17.4 ± 0.9	1*	14.5 ± 1.8
	Spring	-	15.7 ± 1.9	1*	10.6 ± 2.4
O2 (%)	Summer	-	12.8 ± 1.9	1*	12.5 ± 1.2
	Autumn	-	14.3 ± 1.9	1*	12.6 ± 2.4
	Annual	-	15.1 ± 2.4	1*	12.6 ± 2.4

* Theoretical oxygen concentration based on literature values were used.

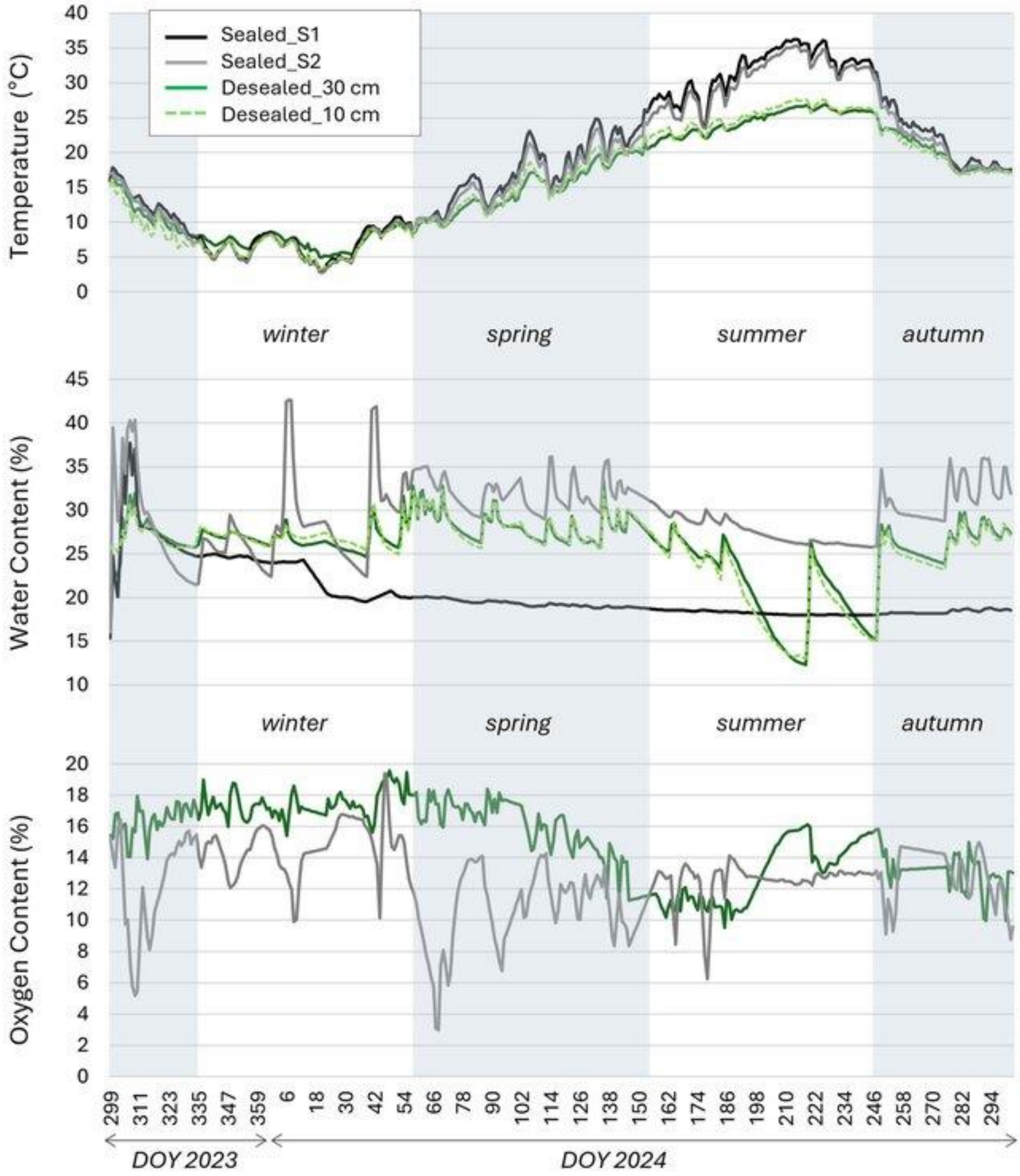


Figure 2. Pedoclimatic parameters curves during the monitoring period, divided by soil cover groups and desealed soil depth.

Table 2. Seasonal and annual effect size and Cliff's delta (δ) values across the soil cover groups. Cliff's δ interpretation: negligible (|δ| < 0.147), small (0.147 ≤ |δ| < 0.330), medium (0.330 ≤ |δ| < 0.474) and large (|δ| ≥ 0.474).

Parameter	Period	D_{30} – S1			D_{30} – S2			S2 – S1		
		Effect size	**Cliff's δ**	**Interpretation**	**Effect size**	**Cliff's δ**	**Interpretation**	**Effect size**	**Cliff's δ**	**Interpretation**
T (°C)	Winter	0.4	0.139	Negligible	0.8	0.235	Small	-0.3	-0.104	Negligible
	Spring	-2.7	-0.359	Medium	-1.7	-0.251	Small	-1	-0.149	Small
	Summer	-7.2	-0.932	Large	-5.9	-0.829	Large	-1.2	-0.220	Small
	Autumn	-1.7	-0.145	Negligible	-0.9	-0.057	Negligible	-0.8	-0.097	Negligible
	Annual	-2.8	-0.144	Negligible	-1.9	-0.093	Negligible	-0.8	-0.052	Negligible
SWC (%)	Winter	4.5	0.993	Large	-1.6	-0.146	Negligible	6	0.805	Large
	Spring	8.8	1	Large	-3.8	-0.877	Large	12.6	1	Large
	Summer	3.2	0.432	Medium	-6.3	-0.845	Large	9.5	1	Large
	Autumn	4.6	0.547	Large	-3.8	-0.568	Large	8.3	0.762	Large
	Annual	5.2	0.706	Large	-3.9	-0.525	Large	9.1	0.914	Large
O2 (%)	Winter	16.4*	1*	Large*	2.9	0.914	Large	13.5*	1*	Large*
	Spring	14.7*	1*	Large*	5.1	0.893	Large	9.6*	1*	Large*
	Summer	11.8*	1*	Large*	0.3	0.025	Negligible	11.5*	1*	Large*
	Autumn	13.3*	1*	Large*	1.8	0.360	Medium	11.6*	1*	Large*
	Annual	14.1*	1*	Large*	2.5	0.530	Large	11.6*	1*	Large*

* Theoretical oxygen concentration based on literature values were used.

Table 3. Effect size and Cliff's delta (δ) values between the treatments applied to de-sealed plots (CoA = Compost addition; VgT = Vegetation type). Cliff's delta interpretation: negligible ($|\delta| < 0.147$), small ($0.147 \le |\delta| < 0.330$), medium ($0.330 \le |\delta| < 0.474$) and large ($|\delta| \ge 0.474$).

Parameter	Treatment	Effect size	Cliffs_delta	Interpretation
T (°C)	CoA	0.1	0.019	Negligible
	VgT	-0.2	-0.031	Negligible
SWC (%)	CoA	-0.5	-0.016	Negligible
	VgT	1.2	0.057	Negligible
O2 (%)	CoA	0.1	0.047	Negligible
	VgT	-3.9	-0.824	Large

Soil Properties

At time zero (October 2023), the de-sealed soil collected from the 30 cm layer immediately beneath the RA cover showed a pH range of 6.7–7.2, organic carbon content between 0.43% and 0.55%, total nitrogen between 0.05% and 0.06%, and a C:N ratio of 7.8–8.7. Over the following year, pH exhibited a non-linear trend: starting from neutral values in October, it rose in November after RA deposition, returned to neutrality in spring, increased again in summer, and finally declined to 7.2 in autumn (Figure 3). Similar seasonal oscillations were observed in $CaCO_3$ content, although these variations were not statistically significant.

Organic carbon and total nitrogen both increased during the monitoring period, but differences among sampling dates were not significant. However, the C:N ratio indicated that t_0 and t_2 differed significantly from t_5, when higher SOC and lower nitrogen levels produced a marked rise in the C:N ratio.

Percolation Water

Metal concentrations in percolation water collected from the lysimeters are reported in Table 4. Control values, representing leachate from de-sealed soil, were generally low for Pb, Cu, Ni, Cr, and Zn, with the only exceptions being Pb on 7 March 2024 (0.24 mg L^{-1}) and Ni on 6 November 2023 (0.09 mg L^{-1}).

The net metal release from the RA layer, calculated as the difference between the mean concentrations in percolation water from the four experimental plots and the corresponding control values, was also generally low. The highest releases occurred for Pb (0.14 ± 0.13 mg L^{-1}) and Ni (0.06 ± 0.06 mg L^{-1}) at the first sampling. Afterward, RA-derived contributions decreased for all monitored metals, approaching zero by the final sampling date (≤ 0.01 ± 0.01 mg L^{-1}).

Bacterial Community Composition

After quality filtering and removal of non-bacterial ASVs, a total of 521,131 sequences were obtained, ranging from 1,355 to 96,265 per sample. Data from three samples (D1 in April 2024, D3 in July 2024, and D4 in October 2024) were unavailable due to accidental loss.

Characterization of the bacterial community showed that much of the soil diversity remains unknown: 72% of ASVs were unclassified at the genus level, and 47% at the order level. Caryophanales and Gemmatimonadales were the most abundant orders across all experimental plots throughout the year, followed by Micromonosporales and Hyphomicrobiales (Figure 4).

Occasional plot-specific differences appeared in December 2023 and April 2024, when D4 and D2, respectively, showed higher abundances of Burkholderiales (and Cytophagales in D4) compared with other samples, including those from the same plots at other times. Overall, no consistent temporal trends or clear spatial differentiation among plots were observed.

Soil, pedoclimate and Bacterial Communities

The first two principal components of the PCA (Figure 5a) accounted for 40.9% of the total variance (PC1: 22.7%, PC2: 18.2%). As shown in Figure 5b, SOC and the C:N ratio were the main drivers of PC1 and PC2, followed by SWC and N. In contrast, pH, T, and O_2 mainly contributed to the third component (PC3), which explained an additional 16.6% of the variance. The PCA plot indicates that SOC and the C:N ratio were positively correlated with pH and temperature. Regarding bacterial orders, Xanthomonadales were closely associated with high SOC, high C:N ratios, and elevated T, while Gemmatimonadales, Micromonosporales, and Eubacteriales correlated positively with SWC and O_2.

Linear mixed model results (Table 5) showed that Hyphomicrobiales abundance was significantly influenced by SOC, N, and the C:N ratio. Soil pH affected only Gemmatimonadales, whereas carbonate content influenced Eubacteriales and Micrococcales. Among pedoclimatic factors, T significantly affected only Eubacteriales, and SWC affected Xanthomonadales, while no significant relationships were observed with O_2. Finally, Solirubrobacterales and Micrococcales were the only orders significantly affected by ‘days after de-sealing,’ with the abundance of Micrococcales further influenced by N.

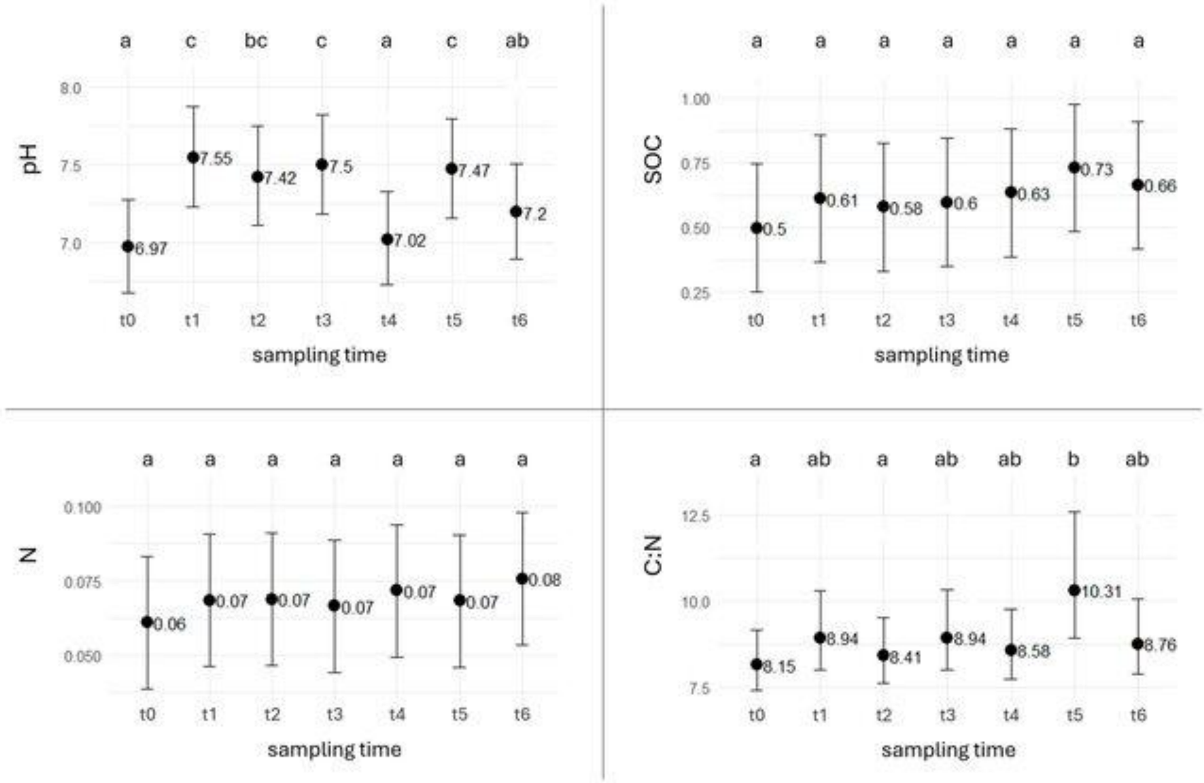


Figure 3. Plots of estimated marginal means (EMMs). Evolution of soil characteristics during time. Different letters indicate statistically significant differences.

Table 4. Metal concentrations in percolation water: control values and mean net release from the RA layer.

Sampling days	Control values (mg L^{-1})					From RAs (mg L^{-1})				
	Pb	Cu	Ni	Cr	Zn	Pb	Cu	Ni	Cr	Zn
6 November 2023	0.07	0.03	0.09	0.00	0.02	0.14 ± 0.13	0.03 ± 0.02	0.13 ± 0.06	0.00 ± 0.00	0.02 ± 0.00
31 January 2024	0.06	0.01	0.03	0.00	0.01	0.02 ± 0.02	0.07 ± 0.04	0.09 ± 0.08	0.00 ± 0.00	0.02 ± 0.03
7 March 2024	0.24	0.04	0.02	0.06	0.01	-0.03 ± 0.05	0.03 ± 0.02	0.03 ± 0.06	0.02 ± 0.02	0.03 ± 0.05
11 April 2024	0.00	0.04	0.04	0.04	0.02	0.00 ± 0.00	0.03 ± 0.02	0.05 ± 0.06	0.00 ± 0.01	0.02 ± 0.04
08 May 2024	0.00	0.04	0.06	0.03	0.02	0.00 ± 0.00	0.03 ± 0.02	0.05 ± 0.05	0.00 ± 0.01	0.00 ± 0.01
05 June 2024	0.00	0.04	0.04	0.00	0.01	0.00 ± 0.00	0.03 ± 0.02	0.05 ± 0.03	0.00 ± 0.00	0.02 ± 0.03
24 July 2024	0.00	0.03	0.05	0.00	0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.01

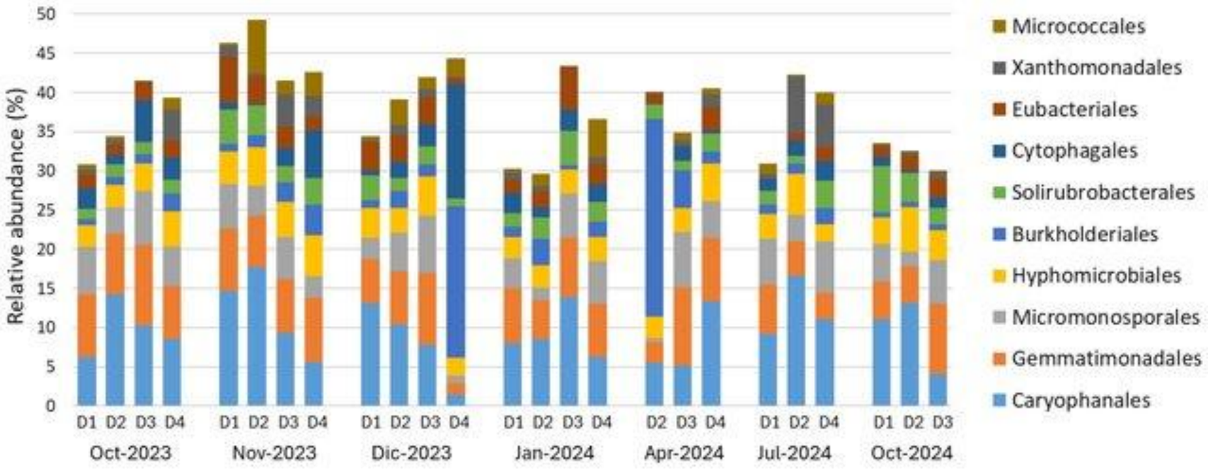


Figure 4. Relative abundance of the ten most abundant bacterial orders. D1, D2, D3 and D4 represent the experimental plots. Due to accidental reasons, data from D1 in April 2024, D3 in July 2024 and D4 in October 2024 are missing.

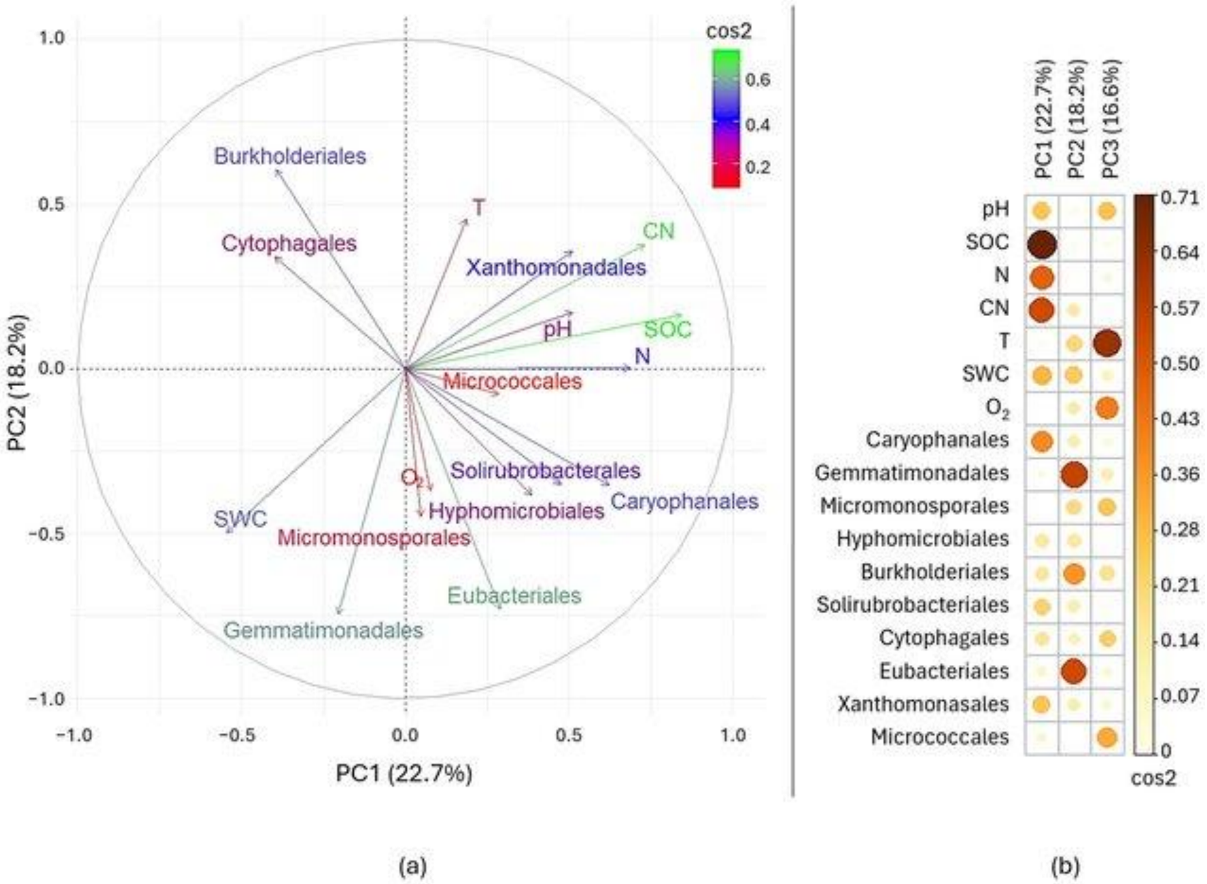


Figure 5. (a) PCA showing the relationships among the ten most abundant bacterial orders, pedoclimatic factors (T, SWC, O2) and soil properties (pH, SOC, N, C:N). **(b)** Quality of representation (Cos2) of each variable to PC1, PC2 and PC3 separately.

Table 5. Results of linear mixed models: *p*-values of the significant predictors (previously selected for each order with the stepwise model) that affected the abundance of bacterial orders. R^2m and R^2c are respectively the marginal and the conditional R-squared of each model. R^2m represents the variance explained by fixed effect only, while R^2c represents the variance explained by both the fixed and random effects.

Order	**Day**	**pH**	**$CaCO_3$**	**SOC**	**N**	**CN**	**T**	**SWC**	**O2**	**R^2m**	**R^2c**
Caryophanales					0.059					0.14	0.31
Gemmatimonadales		0.031*								0.22	0.47
Micromonosporales				0.374		0.732				0.05	0.57
Hyphomicrobiales				0.020*	0.016*	0.031*	0.051		0.098	0.35	0.35
Burkholderiales				0.097						0.09	0.49
Xanthomonadales	0.103	0.207					0.113	0.011*		0.52	0.52
Solirubrobacterales	0.015*						0.055			0.25	0.25
Cytophagales			0.052			0.065		0.070	0.257	0.19	0.39
Eubacteriales			0.049*				0.002*			0.38	0.38
Micrococcales	0.023*		0.003*		0.012*					0.47	0.47

* *p*-values statistically significant.

DISCUSSION

De-sealing Effects on Pedoclimate Factors

This experiment demonstrated that de-sealing strongly affected the soil microclimate. DE-sealed soils showed lower mean temperatures than sealed soils, particularly during summer. This difference results from the warming effect of impervious surfaces, which retain more radiation and accumulate more heat than bare soil [28]. Removing the pavement restores direct soil–atmosphere heat exchange and allows dissipation through conduction, evaporation, and, once vegetation establishes, evapotranspiration. The improved heat exchange in de-sealed soil was evident in two ways: larger daily temperature fluctuations, reflecting responsiveness to meteorological conditions, and reduced seasonal thermal extremes. Seasonal thermal buffering is especially important in summer, when de-sealed soils maintained temperatures around 24–25°C (mean values at 10 cm and 30 cm depth, respectively), compared with 30–31 ∘C in sealed soils (mean values at 30 cm depth in S2 and S1, respectively). Since lower soil temperatures help reduce surface air temperatures, these differences are relevant for mitigating the urban heat island effect.

Soil moisture dynamics showed that de-sealing enhances water infiltration and regulation. At the same time, de-sealed soils were more vulnerable to summer drought, confirming their strong response to meteorological drivers. In this study, partially sealed soils exhibited the highest water content and retention. This pattern likely reflects water infiltration through pavement cracks or preferential pathways that redirect lateral rainfall, leading to localized water accumulation under the sealed layer, where water loss occurs mainly through limited evaporation [29,30]. By contrast, water content in well-sealed soils decreased gradually and independently of rainfall or weather conditions, demonstrating that these soils cannot receive or store rainwater and thus cannot contribute to water regulation functions [31,32]. De-sealing therefore plays a key role in restoring soil water regulation by reestablishing exchanges between the pedosphere, biosphere, and atmosphere.

Due to the malfunction of the S1 oxygen sensor, a literature-based value of 1% [22–24] was used to model a perfectly sealed soil scenario. Other studies have reported oxygen concentrations beneath pavements of 2–5% [33,34], reflecting the many factors influencing soil oxygen, including temperature, moisture, texture, compaction, respiration, pavement type, and sealing conditions [30]. It is possible that actual oxygen in S1 exceeded 1%. However, the very large effect size (Cliff's $\delta = 1.0$) between de-sealed and S1 indicates that even if S1 oxygen ranged from 2–5%, de-sealed soils would still show substantially higher oxygen availability. The exact magnitude of the increase remains uncertain, with reliable observations limited comparisons between de-sealed (D) and partially sealed (S2) soils. Significantly higher oxygen in de-sealed soils confirmed that de-sealing restores more aerobic conditions. Enhanced aeration, in turn, supports microbial activity, root respiration, and nutrient cycling, all of which improve soil quality and function.

Despite this increase, a temporary seasonal decline in soil oxygen occurred in de-sealed plots during summer, likely due to higher temperatures promoting microbial and root respiration [35,36]. Oxygen levels in de-sealed soils were also influenced by vegetation: mixed grasslands consistently showed higher oxygen than sorghum monocultures, reflecting the effects of root morphology and chemistry on respiration and aeration [37]. Experimental treatments in de-sealed plots had no significant effects on soil temperature or moisture.

Our observations of summer cooling and enhanced moisture regulation are consistent with another study in Italy on permeable pavements, which reported surface temperature reductions of 3–5°C and improved infiltration compared with impervious layers [30]. Unlike most previous work, which focused on surface conditions, our monitoring at multiple soil depths and continuous oxygen assessment provides novel insights.

Temporal changes in soil properties and RA effects

While de-sealing immediately affected the soil microclimate, no significant changes in soil properties were observed over the year, reflecting the slow pace of pedogenesis. Small increases in organic carbon, total nitrogen, and cation exchange capacity were recorded, indicating a gradual accumulation of organic matter and enhanced soil buffering. The C:N ratio showed a significant increase in July 2024, likely driven by seasonal inputs of plant residues, roots, and exudates, or by varying decomposition rates mediated by microbial communities [38]. These findings align with the slow pedogenesis reported by Maienza et al. (2021) [6], who observed notable improvements in soil chemical properties 2–3 years after de-sealing. Similarly, Renella (2020) [12] found that microbial activity responded within 6–12 months, while chemical properties remained largely stable during the first year. Together, these studies suggest that biological recovery precedes chemical changes, with 1–2 years representing an early stage in a multi-year restoration trajectory.

Soil pH and carbonate content showed seasonal variability. In the early phase of the experiment, the presence of moisture likely enhanced the dissolution of alkaline substances derived from concrete-based RAs, such as calcium hydroxide [39], temporarily increasing soil pH through the release of OH^- ions. As the experiment progressed, the progressive depletion of the most reactive alkaline compounds and the increasing dominance of less soluble carbonate phases may have contributed to a partial stabilization of pH values, with $CaCO_3$ exerting a buffering effect typical of carbonate-rich systems [39,40]. The higher pH values observed during summer may be influenced by reduced leaching under drier conditions and by concentration effects related to lower soil moisture. At the same time, although microbial respiration likely increased with temperature, as suggested by declining O_2 levels and presumably higher CO_2 production, the potential acidifying effect of microbial activity was likely outweighed by the buffering capacity of carbonates and basic cations. Root activity and exudates may also have contributed to summer pH fluctuations, but differences among vegetation–compost combinations and the lack of plot replicates prevented quantitative assessment. Autumn rainfall then likely flushed accumulated bases, returning pH to near-neutral values.

However, given the limited number of measurements and the intrinsic heterogeneity of the substrate, part of the observed seasonal variability may reflect sampling variability rather than fully distinct geochemical processes.

Monitoring of metals in percolation water showed only minimal release of Pb and Ni at the start of the experiment. These metals can originate from various components of construction and demolition waste, including plaster, mortar, ceramics, and red bricks within the RAs [41–43]. Their concentrations declined over time to levels comparable with control soil, indicating that RA application did not compromise percolation water quality.

Overall, no adverse effects of RAs, such as excessive alkalinization with reduced nutrient availability and biological activity, heavy metal contamination, or phytotoxicity, were observed. pH remained within a neutral to slightly alkaline range (7.0–7.6), suitable for most biological processes. Heavy metal release was minimal and transient, decreasing to control levels within months. Organic matter increased gradually, bacterial communities showed no signs of stress or compositional collapse due to RAs, and vegetation established successfully without nutrient deficiency or toxicity symptoms.

Nevertheless, the chemical and physical properties of RAs can vary depending on source materials and production methods. This variability may influence the release of pollutants, trace elements, and emerging contaminants. Further research is needed to evaluate the chemical behavior and long-term environmental risks of different RA types.

Bacterial community composition and evolution

The high percentage of unclassified taxa observed in the bacterial communities highlighted the still limited knowledge of soil biodiversity [13]. Overall, bacterial orders from Pseudomonadota (29.3 ± 6.6 %), Actinomycetota (17.5 ± 4.7 %) and Bacillota (13.1 ± 5.1 %) phyla were dominant. They are ubiquitous in soils, and known to be involved in the degradation of organic matter and carbon cycling processes [44].

The overall impact of the de-sealing intervention on bacterial community evolution was limited, since no major trends could be observed. Particularly, the high abundance of several subdominant orders of the Actinomycetota phylum, such as Micromonosporales and Solirubrobacterales, which is known to be mainly represented by aerobic members [44], indicates an overall prevalence of aerobic metabolisms. Notably, linear mixed models indicated that none of the most abundant orders was significantly affected by the soil oxygen content, and only two of them were significantly influenced by the 'days after de-sealing'. The lack of a significant relationship with soil oxygen and time since de-sealing may reflect the fact that the soil was already in aerobic conditions at the beginning of the experiment, at least partially. The co-occurrence of several orders of aerobic Actinomycetota and of typically anaerobic bacteria, such as the order Eubacteriales and members of class Deltaproteobacteria, mainly unclassified, may suggest the existence of mixed aerobic/anaerobic conditions [44]. This could be due to a rapid increase in oxygen concentrations and microbial activity occurring within the first 48 hours after de-sealing (time prior to the initial sampling) or, more likely, to uncomplete sealing conditions, which allowed to maintain relatively high oxygen levels in soil, similar to those observed in the partially sealed soil (S2), even before the de-sealing intervention. The absence of a clear temporal trend in bacterial community evolution, despite the observed fluctuations in pH and C:N ratio values, may indicate the occurrence of a relatively stable and robust community, already ecologically adapted to local soil conditions. Prolonged monitoring will be essential to highlight possible shifts due to the long-term effects of de-sealing and/or the different experimental treatments applied.

During the monitored period, a few differences in bacterial community composition were observed among the de-sealed plots, but apparently not related to seasonality or to the different experimental treatments. In contrast, the Principal Component Analysis (PCA) highlighted that the distribution of bacterial orders was strongly influenced by pedological and climatic variables. The variables that drove the major differences in bacterial community composition were SOC and the C:N ratio, followed by soil water content and total nitrogen. This is in contrast with literature, which often indicates pH as the main factor influencing bacterial community structure in soils [13,46]. However, although pH and soil temperature contributed only marginally to the first two principal components, they showed a positive correlation with C:N ratio and SOC, suggesting a possible combined effect on bacterial community structure. In addition, linear mixed models confirmed that the most abundant bacterial orders varied more according to soil characteristics than to pedoclimatic factors.

Such dependence may be regarded as a proxy of a differential involvement of the main bacterial populations in soil biogeochemical cycles. Particularly, Hyphomicrobiales were positively correlated with SOC, C:N ratio and total nitrogen content. Members of this order are nitrogen fixers and can establish endosymbiotic nitrogen fixation relationships with leguminous roots [46,47]. Their increased abundance can therefore be considered as a proxy of increased soil functionality, since they can boost both carbon and nitrogen cycles. Micrococcales were positively correlated to total nitrogen and $CaCO_3$ content, while Gemmatimonadales were negatively correlated with pH. The abundance of the latter has already been shown to be constrained by pH values diverging from neutrality [48]. Due to their active involvement in carbon and nitrogen cycling, both orders are commonly regarded as indicators of soil multifunctionality, and they are generally enriched under appropriate agronomic practices [49–51]. All together, these results suggest that many of the dominant bacterial populations in soils are actively involved in key soil functions, such as carbon storage and nitrogen fixation, and their relative abundance may be modulated by fluctuations in soil properties. Therefore, after a de-sealing intervention, monitoring of such parameters is essential to timely observe the onset of environmental conditions that could impair microbial activity, thus reducing the targeted soil functional recovery. Particularly, it is crucial to monitor pH shifts, since abrupt and strong pH increases may rapidly lead to a depletion of key microbial taxa with consequent loss of soil functions.

Although apparently less impacting on bacterial populations than soil characteristics, pedoclimatic factors, and especially SWC, also played a role in bacterial community assembly. In fact, the dominance of drought tolerant and oligotrophic orders (Caryophanales, mainly represented by families Bacillaceae and Paenibacillaceae, and Gemmatimonadales) aligns with the sandy, relatively nutrient-poor soil and dry summer conditions [48,52,53]. Furthermore, the abundance of Xanthomonadales, mainly belonging to genera *Lysobacter* and *Arenimonas*, was negatively related to soil water content. Several species of *Arenimonas* were previously isolated from saline and alkaline soils [54,55], and their abundance was increased under mixed irrigation with brackish and reclaimed water [56]. Members of genus *Lysobacter* are generally able to produce a wide range of metabolites with activity against other bacteria, fungi and nematodes, and were found to be particularly abundant in disease suppressive soils [57]. Therefore, members of this order may have the ability to cope with stress conditions, especially due to drought, alkalinity, and the presence of plant pathogens, thus contributing to improving soil properties.

CONCLUSIONS

This study provides one of the most comprehensive assessments of de-sealing outcomes conducted in Europe, addressing critical knowledge gaps. Although direct comparisons with other European projects are limited by the scarcity of soil focused field studies, our results align with the available evidence. Pedoclimatic improvements, such as enhanced soil aeration, water regulation, and heat exchange capacity, occurred rapidly after de-sealing, creating favorable microclimatic conditions for biological activity.

Our integrated assessment, which documented pH dynamics, metal leaching, soil property changes, and microbial community responses, also shows that properly characterized concrete-based RAs do not hinder soil restoration in field conditions. At the same time, their use supports circular economy practices by reducing both economic and environmental costs. The minimal pH fluctuations, transient and minor metal release, maintained bacterial diversity, and successful vegetation establishment collectively demonstrate the environmental safety of this approach. However, the effects of RAs on soil and water depend strongly on their composition and the environmental context. Therefore, our findings are representative of the specific experimental site and the RA type used.

The relatively short monitoring period did not capture long-term cumulative effects of de-sealing and RA addition. Pedoclimatic improvements appear structurally stable, but chemical and biological soil development occurs over multiple years or decades. Metal release from RAs is expected to remain low under alkaline conditions, yet uncertainties remain regarding their long-term chemical behavior and degradation pathways. Multiyear monitoring (5–10 years) that integrates pedoclimate, chemistry, microbial and faunal communities, vegetation, and leachate is essential to confirm these preliminary findings and to disentangle the effects of seasonal fluctuations from those induced by de-sealing and RA addition.

The limited comparability across European de-sealing projects highlights the need for coordinated research with standardized monitoring protocols and common indicators. Such harmonization would allow systematic comparisons across sites, climates, RA types, and management practices. The resulting evidence could then be used to optimize de-sealing and restoration strategies, promoting soil development and functional recovery more effectively.

Author Contributions: Conceptualization, G.M., R.C. and C.F.; data curation, G.M., R.C., I.G. and C.F.; formal analysis, G.M., R.C., F.P. and I.G.; investigation, G.M., R.C. and C.F.; methodology, G.M., R.C. and C.F.; project administration, G.M., R.C. and C.F.; resources, G.M., I.G. and C.F.; software, R.C.; supervision, C.F.; validation, R.C.; visualization, G.M. and R.C.; writing—original draft, G.M.; writing—review & editing, G.M., R.C., F.P., I.G. and C.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially 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. Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP H43C22000530001, Project title "National Biodiversity Future Center—NBFC.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Raw DNA sequences were submitted to Sequence Read Archive (SRA-NCBI), with accession numbers SAMN52863942-SAMN52863978, BioProjects PRJNA1347647 (Submission ID: SUB15724367, release date: 15 November 2025) and PRJNA1108077. Other raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: The authors would like to thank the Centro Forestazione Urbana of the Italia Nostra Milano Nord association, which managed the Parco di Porto di Mare area, for technical and financial support in the de-sealing operations, as well as in the management of demolition waste and recycled aggregates.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A. Sensor Data Processing

TEROS 11 sensors use a thermistor to measure temperature [°C]. The measurement is optimized to be accurate when the sensor is fully buried in soil and the value does not need to be converted or corrected. The same sensors use an electromagnetic field to measure the dielectric permittivity of the soil, i.e. the ability of soil to hold an electric charge, strictly linked to the soil moisture. The raw value of dielectric permittivity (RAW) is then converted to percentage volumetric soil water content (SWC) by the following calibration equation for mineral soil:

$$SWC = (3.879 \times 10\text{-}4) \times RAW - 0.6956) \times 100 \qquad (1)$$

Apogee SO-110 series oxygen sensors are galvanic cell type sensors. They operate by electrochemical reaction of oxygen with an electrolyte, which consumes a small amount of oxygen and produces an electrical current. The output is an analog voltage that is linearly proportional to the absolute oxygen concentration. A linear function is used to derive a calibration factor and convert voltage output to the oxygen partial pressure:

$$O_{2m} = CF \times mV_m - Offset \qquad (2)$$

$$CF = (0.2095 \times P_B) / (mV_C - mV_0) \qquad (3)$$

where O_{2m} is the partial pressure of oxygen measured [kPa], CF the calibration factor [kPa(O_2)/mV], mV_m the measured voltage output [mV], and the sensor Offset is calculated by multiplying CF by mV_0, which is the voltage output under zero oxygen condition (O_2 = 0 kPa O_2 ; $mV_0 = 3\pm2$ mV). The mV_C is the voltage output during the onsite calibration [mV] and P_B is the barometric pressure [kPa] (0.2095 multiplied by P_B equals partial pressure of oxygen under ambient conditions). The calibration was performed by placing the sensor over water in sealed chamber, with ambient air filling the headspace. This setup simulated a 100% humidity environment and represented the recommended calibration procedure for measurements in soils.

However, sensor electronics are affected by temperature; thus, an empirical correction derived from measured data must be applied to the partial pressure of oxygen measured:

$$O_2 = O_{2m} + C_3 \times T_S^3 + C_2 \times T_S^2 + C_1 \times T_S + C_0 \qquad (4)$$

$$C_0 = -(C_3 \times T_C^3 + C_2 \times T_C^2 + C_1 \times T_C) \qquad (5)$$

where T_S is the temperature measured by the sensor itself [°C] and T_C is the temperature measured during the calibration [°C]. C_1, C_2 and C_3 are the correction coefficients for Apogee SO-110 sensors ($C_1 = -6.949\text{x}10^{-2}$; $C_2 = 1.422\text{x}10^{-3}$; $C_3 = -8.213\text{x}10^{-7}$).

Bibliography

1. Fabbri, D.; Pizzol, R.; Calza, P.; Malandrino, M.; Gaggero, E.; Padoan, E.; Ajmone-Marsan, F. Constructed Technosols: A Strategy toward a Circular Economy. *Applied Sciences (Switzerland)* 2021, *11*, doi:10.3390/app11083432.
2. European Environment Agency The European Environment – State and Outlook 2020 – Knowledge for Transition to a Sustainable Europe; Publications Office, 2019; ISBN 9789294800909.
3. European Commission and Directorate-General for Environment *Guidelines on Best Practice to Limit, Mitigate or Compensate Soil Sealing*; Publications Office, 2012;
4. European Commission; Directorate-General for Research and Innovation; Veerman, C.; Pinto Correia, T.; Bastioli, C.; Biro, B.; Bouma, J.; Cienciala, E.; Emmett, B.; Frison, E.A.; et al. *Caring for Soil Is Caring for Life – Ensure 75% of Soils Are Healthy by 2030 for Food, People, Nature and Climate – Report of the Mission Board for Soil Health and Food*; Publications Office, 2020; ISBN 9789276216032.
5. Tobias, S.; Conen, F.; Duss, A.; Wenzel, L.M.; Buser, C.; Alewell, C. Soil Sealing and Unsealing: State of the Art and Examples. *Land Degrad Dev* 2018, *29*, 2015–2024, doi:10.1002/ldr.2919.
6. Maienza, A.; Ungaro, F.; Baronti, S.; Colzi, I.; Giagnoni, L.; Gonnelli, C.; Renella, G.; Ugolini, F.; Calzolari, C. Biological Restoration of Urban Soils after De-Sealing Interventions. *Agriculture (Switzerland)* 2021, *11*, 1–12, doi:10.3390/agriculture11030190.
7. Vieillard, C.; Vidal-Beaudet, L.; Dagois, R.; Lothode, M.; Vadepied, F.; Gontier, M.; Schwartz, C.; Ouvrard, S. Impacts of Soil De-Sealing Practices on Urban Land-Uses, Soil Functions and Ecosystem Services in French Cities. *Geoderma Regional* 2024, *38*, e00854, doi:10.1016/j.geodrs.2024.e00854.
8. Sintesi Di Norme, Linee Guida, Buone Pratiche, Casi Studio in Materia Di Limitazione Di Consumo Di Suolo e Resilienza Urbana al Cambiamento Climatico - Azione A1.3 - SOS4LIFE - Save Our Soil for Life; 2017;
9. Caselli, B.; Ceci, M.; De Noia, I.; Garda, E.; Zazzi, M. Towards the Integration of Soil Desealing in the Urban Areas' Transformation Processes. In; 2024; pp. 286–298.
10. ISTAT – Istituto Nazionale Di Statistica, *Ambiente Urbano – Anno 2023*, Roma, 8 Luglio 2025.
11. Aimar, F. Limiting Soil Sealing and Depaving: Local Actions for Regenerating Public Spaces to Build Green Infrastructures. In; 2023; pp. 127–137.
12. Renella, G. Evolution of Physico-Chemical Properties, Microbial Biomass and Microbial Activity of an Urban Soil after de-Sealing. *Agriculture (Switzerland)* 2020, *10*, 1–11, doi:10.3390/agriculture10120596.
13. Fierer, N. Embracing the Unknown: Disentangling the Complexities of the Soil Microbiome. *Nat Rev Microbiol* 2017, *15*, 579–590, doi:10.1038/nrmicro.2017.87.
14. Le Guern, C.; Prézeau, F.; Clozel, B.; Leduc, T.; Musy, M.; Rodler, A.; Tasca-Guernouti, S.; Béchet, B.; Dufrasnes, E.; Stabile, O.; et al. *Soil Properties and Desealing Strategies: Feedback from the DésiVille Project (France)*; 2022;
15. Adobati, F.; Garda, E. Soil Releasing as Key to Rethink Water Spaces in Urban Planning. *City, Territory and Architecture* 2020, *7*, 9, doi:10.1186/s40410-020-00117-8.
16. Commission Decision of 18 December 2014 Amending Decision 2000/532/EC on the List of Waste Pursuant to Directive 2008/98/EC of the European Parliament and of the Council. *Official Journal of the European Union* 2014.
17. Pacheco, J.; De Brito, J.; Lamperti Tornaghi, M. Use of Recycled Aggregates in Concrete: Opportunities for Upscaling in Europe 2023.
18. European Commission; Directorate-General for Internal Market, I.E. and Sme.; Oberender, A.; Fruergaard Astrup, T.; Frydkjær Witte, S.; Camboni, M.; Chiabrando, F.; Hayleck, M.; Akelytė, R. *EU Construction & Demolition Waste Management Protocol Including Guidelines for Pre-Demolition and Pre-Renovation Audits of Construction Works – Updated Edition 2024*; Publications Office of the European Union, 2024;
19. Coull, M.; Butler, B.; Hough, R.; Beesley, L. A Geochemical and Agronomic Evaluation of Technosols Made from Construction and Demolition Fines Mixed with Green Waste Compost. *Agronomy* 2021, *11*, doi:10.3390/agronomy11040649.
20. Morel, A.; Vidal-Beaudet, L.; Brialix, L.; Lemesle, D.; Bulot, A.; Herpin, S. Evolution of Microclimate Following Small Patch De-Sealing and Revegetation in Urban Context. *Urban Clim* 2025, 61, 102371, doi:10.1016/j.uclim.2025.102371.

21. IUSS Working Group WRB. 2022. World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. 4th Edition. International Union of Soil Sciences (IUSS), Vienna, Austria.
22. Abreu, L.; Lutes Christopher C.; Nichols Eric M. 3-D Modeling Of Aerobic Biodegradation Of Petroleum Vapors: Effect Of Building Area Size On Oxygen Concentration Below The Slab. EPA 510-R-13-002; 2013;
23. Verginelli, I.; Yao, Y.; Wang, Y.; Ma, J.; Suuberg, E.M. Estimating the Oxygenated Zone beneath Building Foundations for Petroleum Vapor Intrusion Assessment. *J Hazard Mater* 2016, *312*, 84–96, doi:10.1016/j.jhazmat.2016.03.037.
24. Technical Guide For Addressing Petroleum Vapor Intrusion At Leaking Underground Storage Tank Sites; Washington, D.C., 2015;
25. D.M. 186/2006 - Min. Dell'Ambiente e Della Tutela Del Territorio - Regolamento Recante Modifiche al Decreto Ministeriale 5 Febbraio 1998 «Individuazione Dei Rifiuti Non Pericolosi Sottoposti Alle Procedure Semplificate Di Recupero, Ai Sensi Degli Articoli 31 e 33 Del Decreto Legislativo 5 Febbraio 1997, n. 22.
26. Nava, V.; Leoni, B.; Arienzo, M.M.; Hogan, Z.S.; Gandolfi, I.; Tatangelo, V.; Carlson, E.; Chea, S.; Soum, S.; Kozloski, R.; et al. Plastic Pollution Affects Ecosystem Processes Including Community Structure and Functional Traits in Large Rivers. *Water Res* 2024, *259*, 121849, doi:10.1016/j.watres.2024.121849.
27. Gandolfi, I.; Canedoli, C.; Rosatelli, A.; Covino, S.; Cappelletti, D.; Sebastiani, B.; Tatangelo, V.; Corengia, D.; Pittino, F.; Padoa-Schioppa, E.; et al. Microbiomes of Urban Trees: Unveiling Contributions to Atmospheric Pollution Mitigation. *Front Microbiol* 2024, *15*, doi:10.3389/fmicb.2024.1470376.
28. Nwakaire, C.M.; Onn, C.C.; Yap, S.P.; Yuen, C.W.; Onodagu, P.D. Urban Heat Island Studies with Emphasis on Urban Pavements: A Review. *Sustain Cities Soc* 2020, *63*, 102476, doi:10.1016/j.scs.2020.102476.
29. Nimmo, J.R. The Processes of Preferential Flow in the Unsaturated Zone. *Soil Science Society of America Journal* 2021, *85*, 1–27, doi:10.1002/saj2.20143.
30. Fini, A.; Frangi, P.; Mori, J.; Donzelli, D.; Ferrini, F. Nature Based Solutions to Mitigate Soil Sealing in Urban Areas: Results from a 4-Year Study Comparing Permeable, Porous, and Impermeable Pavements. *Environ Res* 2017, *156*, 443–454, doi:10.1016/j.envres.2017.03.032.
31. Tóth, G.; Ivits, E.; Prokop, G.; Gregor, M.; Fons-Esteve, J.; Milego Agràs, R.; Mancosu, E. Impact of Soil Sealing on Soil Carbon Sequestration, Water Storage Potentials and Biomass Productivity in Functional Urban Areas of the European Union and the United Kingdom. *Land (Basel)* 2022, *11*, 840, doi:10.3390/land11060840.
32. Recanatesi, F.; Petroselli, A. Land Cover Change and Flood Risk in a Peri-Urban Environment of the Metropolitan Area of Rome (Italy). *Water Resources Management* 2020, *34*, 4399–4413, doi:10.1007/s11269-020-02567-8.
33. Morgenroth, J.; Buchan, G. Soil Moisture and Aeration Beneath Pervious and Impervious Pavements. *Arboric Urban For* 2009, *35*, 135–141, doi:10.48044/jauf.2009.024.
34. Owens, P.R.; Wilding, L.P.; Miller, W.M.; Griffin, R.W. Using Iron Metal Rods to Infer Oxygen Status in Seasonally Saturated Soils. *Catena (Amst)* 2008, *73*, 197–203, doi:10.1016/j.catena.2007.07.009.
35. Schnecker, J.; Baldaszti, L.; Gündler, P.; Pleitner, M.; Sandén, T.; Simon, E.; Spiegel, F.; Spiegel, H.; Urbina Malo, C.; Zechmeister-Boltenstern, S.; et al. Seasonal Dynamics of Soil Microbial Growth, Respiration, Biomass, and Carbon Use Efficiency in Temperate Soils. *Geoderma* 2023, *440*, 116693, doi:10.1016/j.geoderma.2023.116693.
36. Leyrer, V.; Poll, C.; Wirsching, J.; Kandeler, E.; Marhan, S. Warming Persistently Stimulates Respiration from an Arable Soil over a Decade, Regardless of Reduced Summer Precipitation. *Soil Biol Biochem* 2024, *194*, 109439, doi:10.1016/j.soilbio.2024.109439.
37. Lak, Z.A.; Sandén, H.; Mayer, M.; Rewald, B. Specific Root Respiration of Three Plant Species as Influenced by Storage Time and Conditions. *Plant Soil* 2020, *453*, 615–626, doi:10.1007/s11104-020-04619-9.
38. Wuest, S. Seasonal Variation in Soil Organic Carbon. *Soil Science Society of America Journal* 2014, *78*, 1442–1447, doi:10.2136/sssaj2013.10.0447.
39. Ekström, T. Leaching of Concrete : The Leaching Process and Its Effects. Doctoral Thesis (monograph), Lund University, 2003.

40. Bouzar, B.; Mamindy-Pajany, Y. Manufacture and Characterization of Carbonated Lightweight Aggregates from Waste Paper Fly Ash. *Powder Technol* 2022, 406, 117583, doi:10.1016/j.powtec.2022.117583.
41. Yu, D.; Duan, H.; Song, Q.; Li, X.; Zhang, H.; Zhang, H.; Liu, Y.; Shen, W.; Wang, J. Characterizing the Environmental Impact of Metals in Construction and Demolition Waste. *Environmental Science and Pollution Research* 2018, *25*, 13823–13832, doi:10.1007/s11356-018-1632-z.
42. Perry, P.M.; Pavlik, J.W.; Sheets, R.W.; Biagioni, R.N. Lead, Cadmium, and Zinc Concentrations in Plaster and Mortar from Structures in Jasper and Newton Counties, Missouri (Tri-State Mining District). *Science of The Total Environment* 2005, *336*, 275–281, doi:10.1016/j.scitotenv.2004.07.007.
43. Diotti, A.; Perèz Galvin, A.; Piccinali, A.; Plizzari, G.; Sorlini, S. Chemical and Leaching Behavior of Construction and Demolition Wastes and Recycled Aggregates. *Sustainability* 2020, *12*, 10326, doi:10.3390/su122410326.
44. Madigan, M.T.; Bender, K.S.; Buckley, D.H.; Sattley, W.M.; Stahl, D.A. *Brock Biology of Microorganisms*; 16th edition.; 2020;
45. Philippot, L.; Chenu, C.; Kappler, A.; Rillig, M.C.; Fierer, N. The Interplay between Microbial Communities and Soil Properties. *Nat Rev Microbiol* 2024, *22*, 226–239, doi:10.1038/s41579-023-00980-5.
46. Bai, S.; Fan, M.; Wu, M.; Sui, X.; Song, Y.; Jiang, Y.; Meng, H.; Liu, Y.; Wang, X.; Hao, X.; et al. Crop Rotation and Organic Fertilizer Maintains Diversity and Activity of CbbL-Carrying CO2-Fixing Bacteria in Reclaimed Coal Mining Soils. *Eur J Soil Biol* 2025, *126*, 103759, doi:10.1016/j.ejsobi.2025.103759.
47. Zhang, Z.; Sun, J.; Wang, D.; Lin, T.; Yin, Y.; Wang, W.; Wang, Y.; Wang, Z.; Fan, L.; Jiao, X. Effects of Rotation Corn on Potato Yield, Quality, and Soil Microbial Communities. *Front Microbiol* 2025, *16*, doi:10.3389/fmicb.2025.1493333.
48. DeBruyn, J.M.; Nixon, L.T.; Fawaz, M.N.; Johnson, A.M.; Radosevich, M. Global Biogeography and Quantitative Seasonal Dynamics of Gemmatimonadetes in Soil. *Appl Environ Microbiol* 2011, 77, 6295–6300, doi:10.1128/AEM.05005-11.
49. Feng, Y.; Chen, H.; Fu, L.; Yin, M.; Wang, Z.; Li, Y.; Cao, W. Green Manuring Enhances Soil Multifunctionality in Tobacco Field in Southwest China. *Microorganisms* 2024, *12*, 949, doi:10.3390/microorganisms12050949.
50. Jiang, Y.; Zhou, C.; Khan, A.; Zhang, X.; Mamtimin, T.; Fan, J.; Hou, X.; Liu, P.; Han, H.; Li, X. Environmental Risks of Mask Wastes Binding Pollutants: Phytotoxicity, Microbial Community, Nitrogen and Carbon Cycles. *J Hazard Mater* 2024, *476*, 135058, doi:10.1016/j.jhazmat.2024.135058.
51. Jia, R.; Qu, X.; Chen, M.; Xu, Y.; Liu, Z.; Peixoto, L.; Lin, Y.; Guo, Y.; Kumar, A.; Chen, J.; et al. Soil Microbial Variability in Contrasting Grown Flue-cured Tobacco under Long-term Continuous Cropping. *Soil Use Manag* 2025, *41*, doi:10.1111/sum.70063.
52. Mandic-Mulec, I.; Stefanic, P.; van Elsas, J.D. Ecology of *Bacillaceae*. *Microbiol Spectr* 2015, *3*, doi:10.1128/microbiolspec.TBS-0017-2013.
53. Zhu, K.; Jia, W.; Mei, Y.; Wu, S.; Huang, P. Shift from Flooding to Drying Enhances the Respiration of Soil Aggregates by Changing Microbial Community Composition and Keystone Taxa. *Front Microbiol* 2023, *14*, doi:10.3389/fmicb.2023.1167353.
54. Kanjanasuntree, R.; Kim, J.-H.; Yoon, J.-H.; Sukhoom, A.; Kantachote, D.; Kim, W. Arenimonas Halophila Sp. Nov., Isolated from Soil. *Int J Syst Evol Microbiol* 2018, *68*, 2188–2193, doi:10.1099/ijsem.0.002801.
55. Xu, L.; Sun, J.-Q.; Liu, X.; Liu, X.-Z.; Qiao, M.-Q.; Wu, X.-L. Arenimonas Soli Sp. Nov., Isolated from Saline–Alkaline Soil. *Int J Syst Evol Microbiol* 2017, *67*, 2829–2833, doi:10.1099/ijsem.0.002026.
56. Zhao, J.; Wang, J.; Cui, B.; Zhai, B.; Hu, C.; Liu, Y.; Xia, L.; Liu, C.; Li, Z. Mixed Irrigation Affects the Composition and Diversity of the Soil Bacterial Community. *Open Geosciences* 2024, *16*, doi:10.1515/geo-2022-0659.
57. Gómez Expósito, R.; Postma, J.; Raaijmakers, J.M.; De Bruijn, I. Diversity and Activity of Lysobacter Species from Disease Suppressive Soils. *Front Microbiol* 2015, *6*, doi:10.3389/fmicb.2015.01243.

Supplementary materials

List of Figures

Figure S1. Pedological profile P2 in the study area: Eutric Transportic Regosol (Epiloamic, Endoarenic, Ochric).

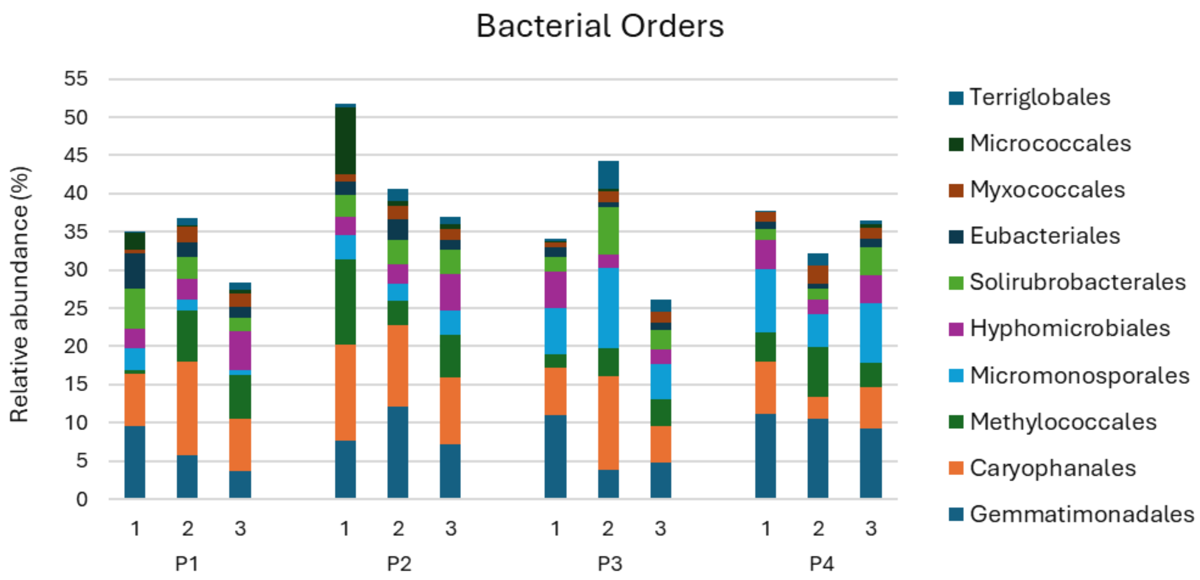


Figure S2. Relative abundance (%) of the ten most abundant bacterial orders, in the first three horizons of each soil profile.

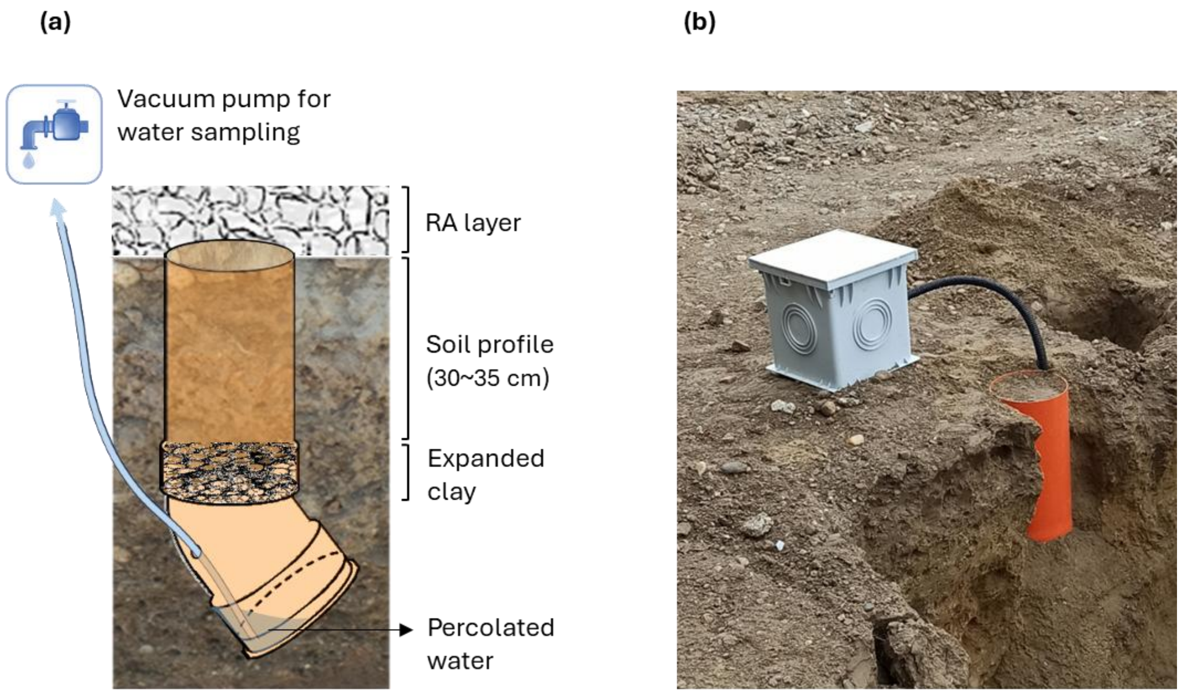


Figure S3. Drainage lysimeter: **(a)** simplified schematic of the lysimeter structure, showing the soil-filled container and the percolated water collection system, and **(b)** lysimeter installation at the Porto di Mare site prior to RA application on the soil surface.

List of Tables

Table S1. Main characteristics of the pedological profiles.

Profile	Horizon	Depth (cm)	pH (in H_2O)	Organic C (%)	Available P (mg/kg)	CEC (cmol(+)/kg)	BS (%)	Text (USDA)
P1	A	0-27	5.8	0.57	65.53	5.05	153	SL
	CB	27-51	6.6	0.43	40.11	5.03	132	SL
	CB/C1	51-92	7.2	0.31	-	-	-	LS
	CB/C2	92-132	7.0	0.14	-	-	-	LS
P2	A	0-18	7.1	0.75	67.06	6.84	132	SL
	Ag	18-46	7.3	0.31	41.70	5.49	126	SL
	C1	46-69	7.4	0.19	-	-	-	LS
	C2	69-115	7.2	0.14	-	-	-	S
	C/CB	115-137	7.3	0.29	-	-	-	SL
P3	A	0-30	6.1	0.54	49.65	6.30	123	SL
	CA	30-58	6.7	0.44	46.27	5.27	139	SL
	CB/C1	58-80	6.9	0.45	-	-	-	SL
	CB/C2	80-115	6.7	0.65	-	-	-	SL
	C	115-140	7.2	0.21	-	-	-	SF
P4	A1	0-20	7.4	0.81	57.02	7.40	140	SL
	A2	20-41	7.4	0.28	33.08	7.88	121	L
	CB1	41-60	7.1	0.42	-	-	-	SL
	CB2	60-84	7.1	0.26	-	-	-	SL
	CB/C	84-105	7.1	0.19	-	-	-	L
	CB/Cg	105-146	7.2	0.18	-	-	-	SL

Table S2. Methods and results of the leaching test performed on the recycles aggregates, with reference limits according to *D.M. n. 186 - Min. dell' Ambiente e della Tutela del Territorio Decreto 05/04/06 - Tab. p. m) All. 3*.

Parameter	Method	Unit	Values	Reference limits
Nitrates	UNI EN ISO 10304-1:2009	mg/l	34,6	50
Fluorides	UNI EN ISO 10304-1:2009	mg/l	< 0,1	1,5
Sulfates	UNI EN ISO 10304-1:2009	mg/l	244	250
Chlorides	UNI EN ISO 10304-1:2009	mg/l	6,32	100
Cyanides (CN)	APAT IRSA CNR 4070	µg/l	< 10	50
Barium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	mg/l	0,028	1
Copper	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	mg/l	0,006	0,05
Zinc	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	mg/l	< 0,001	3
Beryllium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 1	10
Cobalt	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 10	250
Nickel	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	2,61	10
Vanadium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 10	250
Arsenic	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 10	50
Cadmium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 1	5
Total Chromium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 10	50
Lead	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 10	50
Selenium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 1	10
Mercury	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/l	< 0,5	1
Asbestos	DM 06/09/1994 MOCF	mg/l	< 5	30
COD	ISO 15705:2002	mgO_2/l	15	30
pH	UNI EN ISO 10523:2012	-	8,7	5,5 -12,0
Conductivity	UNI EN 27888:1995	mS/cm	1,29	

CHAPTER 3

Restoring Urban Soils after Desealing: Effects of Recycled Aggregates, Compost, and Vegetation on Soil Properties and Microbial Communities

Gaia Mascetti, Francesca Pittino, Isabella Gandolfi, Roberto Comolli and Chiara Ferré

DISAT-Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano Bicocca, Milano, Italia

In preparation

Abstract

Soil desealing is increasingly adopted to contrast land take, improve urban environment and life quality in cities. However, the combined effects of recycled aggregates (RAs), organic amendments, and vegetation establishment on soil restoration after desealing remain poorly understood. This chapter investigates the evolution of soil properties, bacterial communities, and vegetation growth through a field experiment in Porto di Mare, Milan (Italy). Following desealing, the experiment was established using concrete-based RAs derived from pavement demolition waste as a surface layer and growth substrate, combined with compost addition and the sowing of two different vegetation types: sorghum monoculture and mixed ryegrass-clover cover. Soil chemical properties and bacterial communities were monitored over one year, while vegetation development was assessed over six months during the growing season. Results showed that RAs induced transient changes in soil pH and carbonate content, influencing bacterial community composition, which remained distinct in the untreated plots compared to RA-amended ones. The strongest shifts occurred in comparison to time zero, reflecting early microbial responses to soil disturbance and environmental changes. The experimental treatments did not result in significant changes in most soil chemical properties, with only minor variations in soil organic carbon, nitrogen content, and pH, mainly attributable to vegetation type. Bacterial community composition and diversity were primarily driven by seasonal factors rather than experimental treatments. Overall, the results indicate that combining desealing with RAs can support early-stage soil recovery and do not compromise soil quality or vegetation establishment

INTRODUCTION

Soils are fundamental ecosystems that host a vast biodiversity and provide essential functions, including nutrient cycling, water regulation, and biomass production. However, urbanization processes, land-use changes and agricultural practices have led to widespread soil degradation and biodiversity loss, impairing ecosystem services at multiple scales [1,2]. Compaction, erosion, contamination and sealing are some of the main soil threats in Europe and the primary causes of soil degradation in urban environments [3,4].

In particular, artificial soil sealing is a physical form of degradation which consists in permanently covering the soil with impermeable artificial materials [3], which separate soil from other environmental compartments, such as biosphere, atmosphere and hydrosphere [5]. Soil sealing affects the provision of several ecosystem services by reducing water infiltration and biomass production, and leading to habitat fragmentation and biodiversity loss. Currently, land take is recognized as one of the main drivers of biodiversity decline in Europe [6], and soil sealing alone is expected to cause up to a 35% reduction in biodiversity from 2000 to 2030 [3]. This loss of biodiversity, in turn, further compromises most ecosystem functions and their stability over time.

While most past research has focused on aboveground biodiversity, over the last two decades increasing attention has been given to the fundamental role of microorganisms (bacteria, archaea, fungi) in shaping and sustaining terrestrial ecosystem functioning. Their community composition and functional traits, more than species richness alone, regulate biogeochemical cycling and plant-soil interactions [7,8]. These communities contribute to multiple ecological processes and support ecosystems stability, as well as their responses to environmental changes, disturbances, and restoration interventions. Even in urban contexts, soils can sustain remarkably high microbial diversity, and recent evidence demonstrated that desealing interventions can promote microbiome recovery, especially when combined with appropriate substrates and vegetation [9,10].

However, the extreme complexity and heterogeneity of belowground communities, shape by both biotic and abiotic factors across multiple spatial and temporal scales, still limits our full understanding of its structure and dynamics [7]. Advancing this knowledge is crucial for predicting how soil communities respond to disturbances, such as soil sealing, and subsequent restoration interventions, and for developing effective strategies for soil and ecosystem recovery.

As already discussed in Chapter 2, desealing or depaving – the removal of the impermeable surface layers – has recently been recognized as an effective approach to restore soil permeability and implement green infrastructure, thereby enhancing urban resilience to flooding and heatwaves. However, soil desealing also contributes to biodiversity recovery by creating suitable conditions for plants, soil fauna, prokaryotes, and fungi to re-establish. All these organisms are crucial for restoring soil functions, and among them, prokaryotic communities are the main drivers of biogeochemical cycles and nutrient turnover, which sustain soil fertility and multifunctionality [8,9].

Interest and research on the specific role of desealing in soil restoration and multifunctionality are rapidly growing. Recent studies have demonstrated that desealed soils can progressively recover their functions and ecosystem services, especially when combined with soil decompaction, amendment addition and the establishment of vegetation [10–13]. The careful selection of the most appropriate amendments and plant species, based on soil properties, climate, and intended land use, is essential for ensuring the effectiveness of desealing and restoration interventions [14,15].

Another crucial point in desealing interventions is the generation of construction and demolition waste (CDW) from pavement removal. Aligned with circular economy principles, the on-site reuse of these materials – carefully assessed for composition, quality, and contamination risk – represents a suitable approach to reduce disposal and transport costs [14,16]. Moreover, recent studies suggest that CDW and their derived recycled aggregates (RAs) may be cost-effective and promising soil constituent materials [17–19].

This chapter presents the desealing field experiment and one-year monitoring conducted at Porto di Mare, Milan (Italy), partially introduced in Chapter 2. In the previous chapter, we primarily focused on pedoclimatic monitoring following the removal of the sealing layer, and on the assessment of the potential risk of contaminant release in percolating water due to the on-site reuse of RAs. Here, we expand the study area and investigate the influence of RAs, compost, and vegetation type on the temporal evolution of soil properties, bacterial community composition and diversity, and vegetation growth.

MATERIALS AND METHODS

The study area is a brownfield site of approximately 600 m^2, located in the south-east of Milan, Italy (45°25'27.0" N; 9°14'0.9" E). The site was desealed in October 2023 with the collaboration of the Urban Forestry Centre (CFU), the operative center of the national association “Italia Nostra” which currently manages the site. Figures S1 and S2 in the Supplementary Materials illustrate the study area before the desealing intervention and the soil surface after desealing, respectively.

The demolition waste derived from desealing operations and partially from abandoned buildings (CER 17 09 04) [20] was crushed and sieved to 4 cm at the Sereca Sas plants, located approximately 1 km from the experimental site. The resulting recycled aggregates, which were mainly composed of concrete, sand, bricks, and stones, were then evenly spread over the desealed soil surface two weeks after desealing, creating a 15–20 cm thick soil-like layer. Prior to their use in the field, a leaching test was performed on the RAs by the producer [21].
On April 5, 2024, four treatments were established to assess the combined effects of compost addition and vegetation type on soil properties and bacterial community composition. Green waste compost was added to half of the plots, incorporated in the first centimeters of the RA layer, and two types of vegetation were sown: (i) a monoculture of *Sorghum bicolor* and (ii) a mixture of ryegrass (*Lolium perenne, italicum* and *x boucheanum*) and clover (*Trifolium repens, pratense, resupinatum* and *hybridum).* These two vegetation types were selected for their contrasting uses and functional traits, which may influence early soil recovery processes or be affected by RA presence. Mixed grass covers are commonly adopted in urban areas for their low maintenance requirements and rapid soil coverage, whereas *Sorghum bicolor* is a high-biomass annual species widely used for forage, characterized by rapid growth and a deep and well-developed root system, in contrast to the shallow and fibrous roots typical of perennial mixed grasses.
The resulting experimental design is represented in Figure 1, and consisted of four experimental treatments on desealed soil (D1, D2, D3, D4), with three replicates randomly distributed across three areas of the site (I, II, III). The experimental plots measured approximately 4x4 m. Figure S3 in the Supplementary Materials shows the study area as it appeared in April 2024.
A control area (D0), uncovered by the RA layer, was maintained to evaluate soil evolution after desealing in the absence of treatments and further human intervention. Due to operative limitations, the untreated plots differed from the experimental ones in terms of shape and dimensions. During the summer season, the untreated plots were colonized by spontaneous vegetation from the nearby urban park.
In the absence of precipitation, the experimental site was regularly and uniformly irrigated to maintain adequate soil surface moisture and promote vegetation growth. The control area was equally irrigated to ensure that the environmental conditions were comparable to those of the experimental plots.

Soil sampling and Analyses

The evolution of soil features was monitored by periodically sampling the 30 cm layer of the desealed soil immediately beneath the RA layer. Soil site characterization occurred on October 17, 2023, and marked the beginning of the monitoring activities (time zero, t_0).

Soil sampling followed the procedure described in Chapter 2, which involved monthly samplings till January 2024, and quarterly sampling from individual subplots after vegetation establishment in April 2024. Soil samples were then air-dried and sieved for standard laboratory analyses: pH, total carbonates ($CaCO_3$), soil organic carbon (SOC) and total nitrogen (N).

Soil samples for the bacterial community analysis were collected following the same procedure, using nitrile gloves and ethanol-sterilized equipment, and were stored at -20°C. Bacterial communities were analyzed as described in Chapter 2, amplifying the V5–V6 hypervariable regions of the 16S rRNA gene and sequencing the resulting amplicons by MiSeq Illumina. Amplicon Sequence Variants (ASVs) inferring and classification were performed as described by Gandolfi et al. [22].

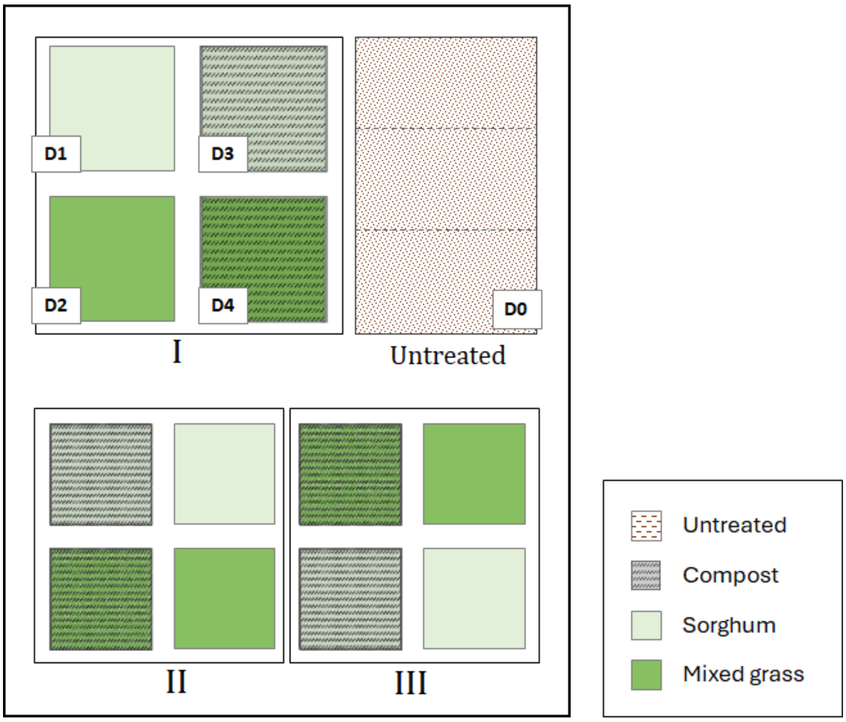


Figure 1. Experimental design showing the untreated control area (D0) and experimental plots: Sorghum (D1), mixed grass (D2), Sorghum with compost (D3) and mixed grass with compost (D4). The experimental plots were randomly replicated three times (I, II, III).

Vegetation monitoring

Vegetation monitoring was carried out exclusively in the sown experimental plots, while spontaneous vegetation in the untreated plots was not included in either the survey or the sampling.

The monitoring was conducted on the half of each experimental plot that was left undisturbed by soil sampling. Vegetation growth was assessed through regular observations throughout the growing season, and biomass production was measured at the maturity of each vegetation type.

In July, floristic surveys were conducted on mixed grass plots, to determine the relative abundance of Gramineae and Leguminosae, by visually estimating the percentage of ground area covered by each plant family across the experimental plot. Then, grass biomass samples were collected from three areas of known surface (18x18 cm) per plot, air dried at room temperature, and weighed. Sorghum biomass was collected in September prior to the harvest, from two areas of known surface (1 m^2) per plot. For each sample, the total fresh biomass and the biomass of two representative plants were recorded. The representative plants were then air-dried and weighed.

Data Analysis

Statistical analyses were performed using RStudio software (version 4.4.2). The effects of desealing, RA addition, vegetation type, and compost amendment on soil chemical properties and bacterial communities were investigated in two experimental phases: (i) during the first three months of the experiment, to assess the short-term effects of desealing and RA addition; and (ii) during the vegetative season, after experimental treatment application, to evaluate the effects of vegetation type and compost amendment.

Linear mixed models (lme function) were used to analyze temporal variations in soil properties. Plots were included as random factors, and separate models were fitted to evaluate the interactions between time and each experimental factor (RAs, experimental treatments, vegetation type, and compost amendment). Spatial independence of residuals was tested with Moran's I, and model validity with the Shapiro–Wilk test. When model assumptions were not verified, variables were appropriately transformed prior to analysis. The most accurate model was selected using the Akaike Information Criterion (AIC). Post hoc Tukey tests on estimated marginal means (EMMs) were used to assess significant differences between treatments and sampling times.

Redundancy analysis (RDA) was performed on Hellinger-transformed ASV relative abundance, to summarize the ASV distribution according to the sampling times and treatments. The effects of these two factors were assessed both in interaction (time × treatment) and as additive terms (time + treatment). Variation partitioning was used to quantify the proportion of variance explained by treatment and time, based on adjusted R^2 values. Pairwise post hoc tests were then applied to detect significant differences between treatments and sampling times, with p-values adjusted using the false discovery rate correction (fdr).

The effects of time and treatments on diversity indices were also assessed. Permutational ANOVA was performed for Shannon and Gini indices (aovp), and generalized linear models were used for ASV richness (glm.nb) and ChaoB (glm). Post hoc Tukey tests were then performed for ASV richness and ChaoB, whereas pairwise permutation tests were applied for Shannon and Gini indices, with p-values adjusted for multiple comparisons using the Benjamini–Yekutieli (BY) correction.

RESULTS

Short-term effects of Desealing and RA addition

Soil properties

At the beginning of the monitoring (t_0), the desealed soils hosting control (D0) and experimental plots (RAs) showed comparable chemical properties (Table 1). The soils were slightly alkaline, with low amounts of $CaCO_3$, and showed similarly low concentrations of soil organic carbon, total nitrogen, and CN ratio.

During the first three months, soil properties did not differ significantly between experimental plots with RAs and those without (Table 1). No significant temporal changes were detected in the untreated plots (D0).

In contrast, in the experimental plots with RAs, pH significantly increased from 6.9 (t_0) to 7.4 (t_1) and 7.3 (t_2 and t_3), accompanied by a significant rise in carbonate content in November (t_1) compared to time zero (Table 1). Organic carbon content was significantly higher in November (t_1) and January (t_3), compared to time zero, with November values also significantly higher than December (t_2). Total nitrogen followed a similar trend, showing a significant increase in November (t_1) and December (t_2) in comparison to time zero. These changes affected the organic carbon to total nitrogen ratio (CN), which was significantly higher in November and January compared to December.

Bacterial communities

The RDA performed using a model including the interaction between time and RA addition (time × RAs) revealed a significant overall effect ($p = 0.013$). However, neither the interaction term nor any of the individual RDA axes were significant; therefore, we focused our analysis on the effects of each factor separately.

The RDA model including time after desealing and RA addition as additive factors (time + RAs; $p < 0.001$) revealed that both the variables significantly contributed to the explained variance (RAs $p < 0.001$ and time $p = 0.02$). However, RDA results (Figure 2a) show that only 3.38% of total variance is explained by the first two axis, and only the first one (2.10%) is significant. The RDA plot shows a clear clustering according to both the presence of RAs (RAs vs D0) and the sampling times. The variation partitioning barplot (Figure 2b) highlighted the small fraction of variance explained by the factors, while the majority (96.67%) remained unexplained.

Post hoc tests revealed significant effects of RA addition on bacterial community composition (Table 2a). The pairwise comparisons of sampling times (Table 2b) showed significant differences between time zero and the later sampling times (t_1, t_2, t_3), with different size effect (fdr = 0.0006, fdr = 0.0273 and fdr = 0.0085, respectively).

Diversity indexes (Shannon, Gini, ASV richness and ChaoB) did not show any significant variation over time or in relation to the presence of RAs.

Table 1. Mean values and standard errors (SE) of soil properties divided by experimental plots (RAs, *n* = *12*) and untreated plots (D0, *n* = *3*), during the first three months of monitoring after desealing and RA addition: October (t_0), November (t_1) and December (t_2) 2023, and January (t_3) 2024. Different letters in the "group" column indicate significant differences among sampling times within each treatment ($p < 0.05$). SOC = soil organic carbon; N = total nitrogen; CN = organic carbon/total nitrogen ratio.

Plot	Time	pH			$CaCO_3$ (g/kg)			SOC (g/kg)			N (g/kg)			CN		
		Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.
D0	t_0	7.10	0.12	a	3.50	2.02	a	0.75	0.02	a	0.078	0.001	a	9.63	0.32	a
	t_1	7.25	0.09	a	4.00	2.31	a	0.89	0.00	a	0.088	0.004	a	10.18	0.50	a
	t_2	7.25	0.09	a	1.50	0.29	a	0.90	0.12	a	0.103	0.005	a	8.63	0.70	a
	t_3	7.30	0.00	a	1.00	0.00	a	0.69	0.04	a	0.090	0.012	a	7.86	0.57	a
RAs	t_0	6.93	0.08	a	3.25	1.60	a	0.62	0.03	a	0.076	0.004	a	8.29	0.34	ab
	t_1	7.40	0.10	b	12.92	2.98	b	0.87	0.07	c	0.093	0.006	b	9.36	0.49	b
	t_2	7.30	0.07	b	3.75	0.75	ab	0.74	0.04	ab	0.094	0.007	b	8.01	0.25	a
	t_3	7.30	0.09	b	4.33	1.44	a	0.78	0.05	bc	0.085	0.005	ab	9.19	0.22	b

Table 2. Posthoc test results of RDA performed on bacterial community composition, including time after desealing and RA addition as additive factors. Pairwise comparisons: (a) between experimental plots with RAs and untreated plot D0; and (b) among sampling times (t_0, t_1, t_2, t_3).

(a)

Post hoc (RAs)	Df	Sum of Sqs	F	p-value	fdr
RAs vs. D0	4	0.05441957	1.462741	1e-04***	1e-04***

(b)

Post hoc (time)	Df	Sum of Sqs	F	p-value	fdr
t_0 vs. t_1	2	0.06034317	1.711707	0.0001***	0.0006**
t_0 vs. t_3	2	0.05208441	1.439343	0.0017*	0.0085*
t_1 vs. t_3	2	0.05802625	1.444746	0.0031*	0.0124.
t_0 vs. t_2	2	0.04947391	1.361858	0.0091*	0.0273.
t_1 vs. t_2	2	0.04813211	1.216496	0.0665.	0.1330
t_2 vs. t_3	2	0.04501304	1.103805	0.1677	0.1677

(a)

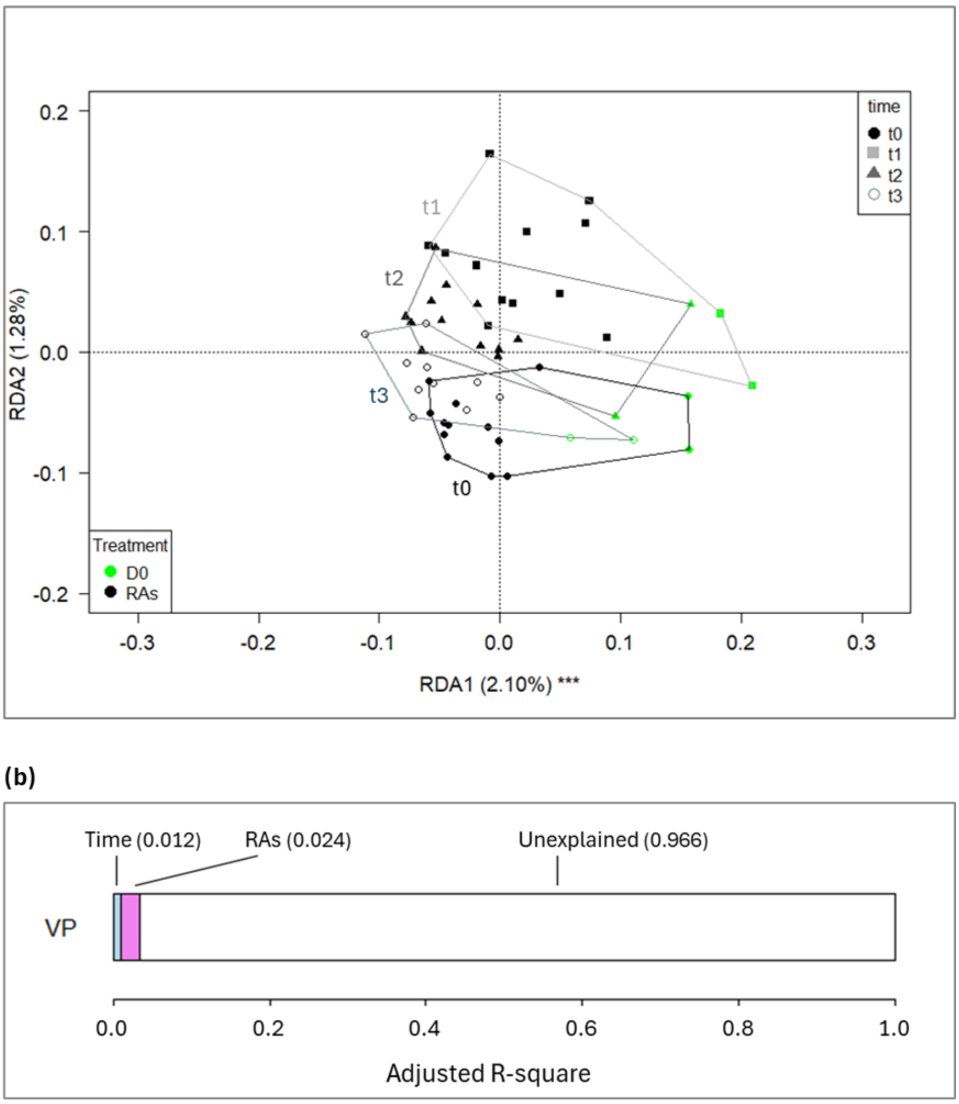


Figure 2. RDA of bacterial community composition, including time after desealing and RA addition as additive factors: **(a)** RDA plot showing ASV distribution according to treatments (D0, RAs) and sampling times (t_0, t_1, t_2, t_3); **(b)** variation partitioning barplot showing the variance explained by each factor (time and RAs addition).

Effects of the experimental treatments

Soil properties

Overall, soil chemical properties did not show significant variations attributable to the experimental treatments. Over time, differences were detected only in total N content, specifically between t_0 and t_5 in the untreated plots D0, and between t_0 and t_4 in D4 treatment (Table S1a). Among treatments, variations in SOC and N were observed in April (t_4), with higher values in D4 than in D3 and D1, respectively, and in July (t_5), with significantly higher SOC in D4 than in D0, and N higher in D2 and D4 than in D0 (Table S2b).

Further statistical analysis of individual factors showed that compost addition did not significantly affect any soil property. In contrast, significant differences were observed between vegetation types (Table 3a), with mixed grass plots showing higher SOC values in t_4 and greater total N content in t_4 and t_5 compared to sorghum plots. Over time, the only significant variation was observed in Sorghum plots, where pH was significantly higher at t_5 than at t_0 (Table 3b).

Table 3. Mean values and standard errors (SE) of soil properties divided by vegetation type (Sorghum and grass) and sampling times: October (t_0) 2023 and April (t_4), July (t_5) and October (t_6) 2024. Different letters in the "group" column indicate significant differences ($p < 0.05$): **(a)** among sampling times within each vegetation and **(b)** among vegetation types within each sampling time.

(a)

Plot	Time	pH			CaCO3 (g/kg)			SOC (g/kg)			N (g/kg)			CN		
		Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.
Sorg.	t_0	6.88	0.12	a	1.33	1.07	a	0.58	0.06	a	0.073	0.007	a	7.92	0.24	a
	t_4	6.93	0.08	ab	0.83	0.92	a	0.58	0.08	a	0.070	0.010	a	8.27	0.53	a
	t_5	7.38	0.19	b	2.33	1.99	a	0.63	0.06	a	0.068	0.006	a	9.38	0.94	a
	t_6	7.12	0.25	ab	0.83	0.57	a	0.65	0.06	a	0.078	0.008	a	8.31	0.35	a
Grass	t_0	6.97	0.12	a	5.17	3.03	a	0.67	0.05	a	0.078	0.006	a	8.65	0.66	a
	t_4	6.90	0.19	a	1.83	0.98	a	0.78	0.07	a	0.089	0.006	a	8.63	0.32	a
	t_5	7.15	0.14	a	6.17	3.59	a	0.76	0.05	a	0.083	0.003	a	9.24	0.71	a
	t_6	7.18	0.15	a	3.33	1.41	a	0.73	0.04	a	0.085	0.003	a	8.66	0.18	a

(b)

Time	Plot	pH			CaCO3 (g/kg)			SOC (g/kg)			N (g/kg)			CN		
		Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.
t_0	Sorg.	6.88	0.12	a	1.33	1.07	a	0.58	0.06	a	0.073	0.007	a	7.92	0.24	a
	Grass	6.97	0.12	a	5.17	3.03	a	0.67	0.05	a	0.078	0.006	a	8.65	0.66	a

t_4	Sorg.	6.93	0.08	a	0.83	0.92	a	0.58	0.08	a	0.070	0.010	a	8.27	0.53	a
	Grass	6.90	0.19	a	1.83	0.98	a	0.78	0.07	b	0.089	0.006	b	8.63	0.32	a
t_5	Sorg.	7.38	0.19	a	2.33	1.99	a	0.63	0.06	a	0.068	0.006	a	9.38	0.94	a
	Grass	7.15	0.14	a	6.17	3.59	a	0.76	0.05	a	0.083	0.003	b	9.24	0.71	a
t_6	Sorg.	7.12	0.25	a	0.83	0.57	a	0.65	0.06	a	0.078	0.008	a	8.31	0.35	a
	Grass	7.18	0.15	a	3.33	1.41	a	0.73	0.04	a	0.085	0.003	a	8.66	0.18	a

Soil bacterial communities

The RDA performed using a model including the interaction between time and treatment (time × treatment) revealed a significant overall effect on ASV distribution ($p = 0.003$). Therefore, since neither the interaction term nor any of the individual RDA axes were significant, we focused our analysis on the effects of each factor separately.

The RDA model including time and treatment as additive factors (time + treatment; $p < 0.001$) showed that both variables significantly contributed to the explained variance ($p < 0.001$). Moreover, the analysis of the individual RDA axes showed that the first four axes (RDA1–RDA4) were statistically significant ($p \leq 0.017$) and the first two, represented in Figure 3a, explained respectively the 5.06% and 4.21% of the total variance. The RDA plot revealed a distinct clustering of D0 samples (untreated plots), whereas samples from the experimental treatments were generally more dispersed and partially overlapping in the RDA space. In addition, samples tended to cluster according to sampling time. Specifically, t_5 (July 2024) and t_6 (October 2024) formed clearly separated clusters, while t_0 and t_4 (October 2023 and April 2024) overlapped. The variation partitioning barplot (Figure 3b) indicated that time and treatment explained only small fractions of the total variation in bacterial community composition (5.7% and 4.3%, respectively), while the shared contribution between the two factors was negligible and the majority of variation (90.7%) remained unexplained.

Post hoc tests on RDA results revealed significant effects of both treatments and sampling times on bacterial community composition. The pairwise comparisons of treatments (Table 4) showed that untreated plots (D0) were significantly different from all the experimental plots (fdr ≤ 0.001), which also showed some significant differences among themselves. In particular, D1 differed significantly from D2 and D3 (fdr ≤ 0.001 and fdr = 0.003, respectively), and D2 was significantly different from D3 (fdr = 0.0036). Comparisons involving D4 were only marginally significant (fdr = 0.0294). Regarding sampling time, all the pairwise comparisons were statistically significant, with the largest differences (fdr = 0.0006) observed between time zero and the later stages t_4 (April 2024) and t_6 (October 2024).

All diversity indices varied significantly over time (ASV richness, p = 0.0079; ChaoB, p = 0.0002; Shannon, p = 0.0148; Gini, p = 0.0045), while no significant effects were observed either for treatment or for the interaction between the two factors. Post hoc comparisons revealed that ASV richness was significantly higher at t_6 compared to t_5 (p = 0.00134), and that ChaoB values were significantly higher at t_6 than at earlier sampling times (p ≤ 0.0057). In contrast, pairwise comparisons showed no significant differences between sampling times for Shannon and Gini indices (Figure 4).

Table 4. Post hoc test results of RDA performed on bacterial community composition, including time after desealing and RA addition as additive factors. Pairwise comparisons among: **(a)** treatments (D0, D1, D2, D3, D4); and **(b)** sampling times (t_0, t_4, t_5, t_6).

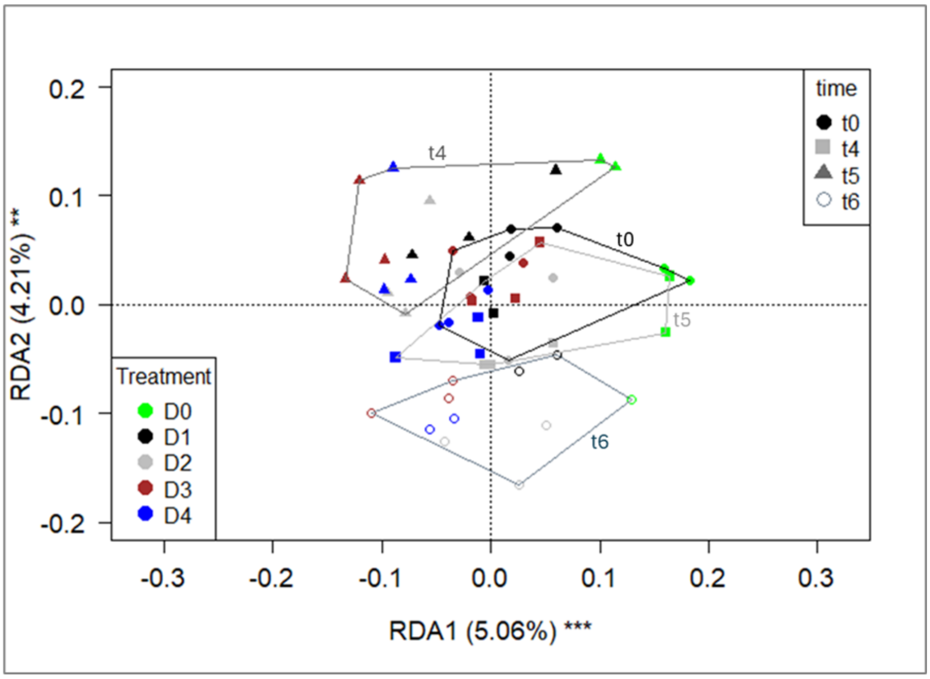
(a)

Post hoc (Tr.)	**Df**	**Sum of Sqs**	**F**	**p-value**	**fdr**
D0 vs. D1	4	0.1730776	1.595224	0.0001***	0.0010**
D0 vs. D2	4	0.1760206	1.464786	0.0001***	0.0010**
D0 vs. D3	4	0.1720098	1.612290	0.0001***	0.0010**
D0 vs. D4	4	0.1648992	1.612116	0.0001***	0.0010**
D1 vs. D3	4	0.1321147	1.394815	0.0001***	0.0010**
D1 vs. D2	4	0.1452669	1.395595	0.0006**	0.0030*
D2 vs. D3	4	0.1330429	1.329619	0.0009**	0.0036*
D1 vs. D4	4	0.1156743	1.219071	0.0075*	0.0225.
D2 vs. D4	4	0.1225244	1.236681	0.0147.	0.0294.
D3 vs. D4	4	0.1102030	1.228607	0.0226.	0.0294.

(b)

Post hoc (time)	**Df**	**Sum of Sqs**	**F**	**p-value**	**fdr**
t_0 vs. t_4	5	0.1386025	1.443359	0.0001***	0.0006**
t_0 vs. t_6	5	0.1370875	1.503018	0.0001***	0.0006**
t_5 vs. t_6	5	0.1499986	1.433333	0.0011**	0.0044*
t_0 vs. t_5	5	0.1230030	1.356454	0.0015**	0.0045*
t_4 vs. t_6	5	0.1523113	1.316506	0.0028**	0.0056*
t_4 vs. t_5	5	0.1390392	1.267435	0.0068**	0.0068*

(a)



(b)

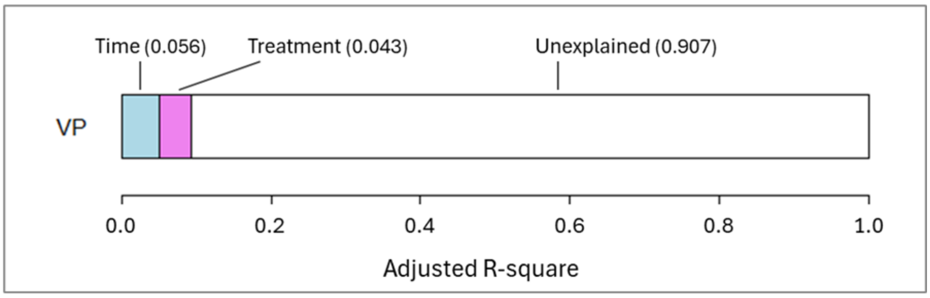


Figure 3. RDA of bacterial community composition, including time after desealing and RA addition as additive factors: **(a)** RDA plot showing ASV distribution according to treatments (D0, D1, D2, D3, D4) and sampling times (t_0, t_4, t_5, t_6); **(b)** variation partitioning barplot showing the variance explained by each factor (time and treatment).

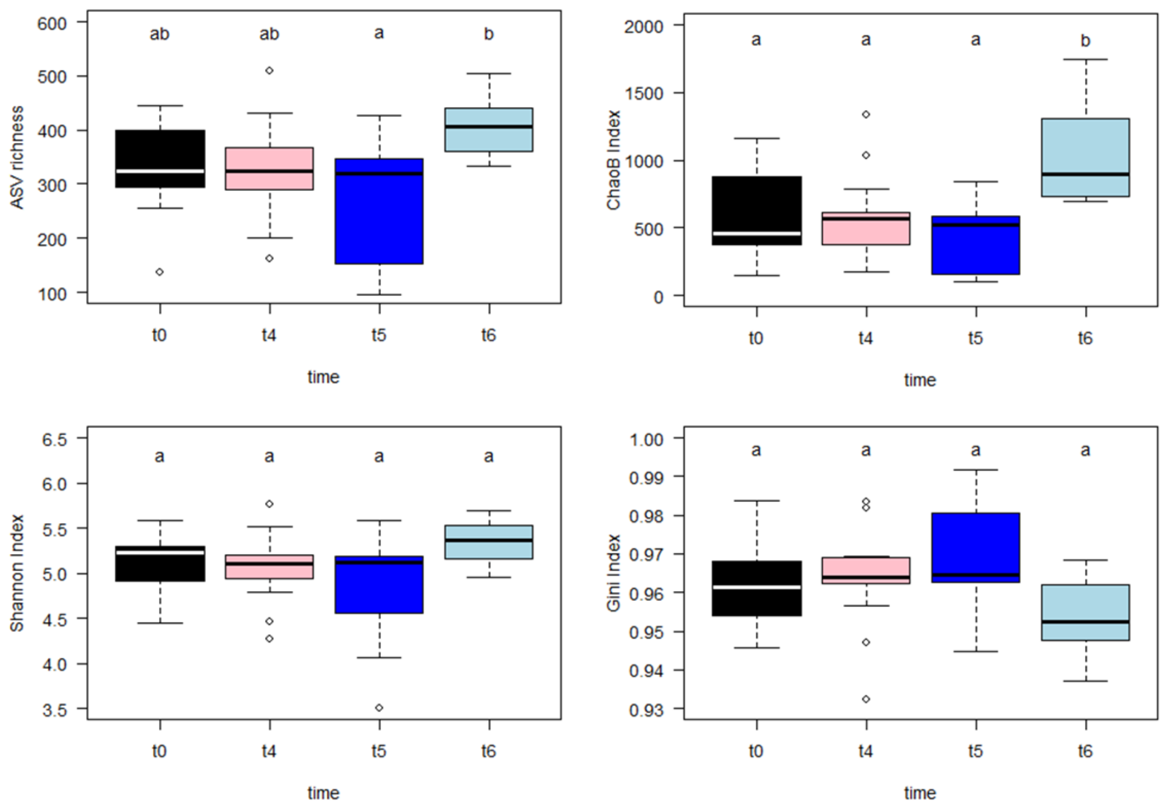


Figure 4. Boxplots of bacterial diversity indices (ASV richness, ChaoB, Shannon, and Gini). Different letters indicate significant differences among sampling times (t_0, t_4, t_5, t_6).

Vegetation growth

One month after sowing, herbaceous species exhibited a greater growth compared to sorghum, and both vegetation types showed a better initial development in plots without compost. However, once germination occurred (Figure S4), compost enhanced vegetation growth in the corresponding plots. The effect of compost on vegetation growth was particularly evident in sorghum plots, where plants exhibited a darker green color and greater height compared to the treatments without compost (Figure S5). Some variability in vegetation growth was also observed within individual plots and among replicates of the same treatment.

The floristic survey conducted in July revealed a higher percentage of Leguminosae than Gramineae in all the plots, except for the first replicate of D2 (Table 5). Moreover, the percentage of Leguminosae increased in the plot with compost (D4). Missing data in the third replicate of D4 are due to a severe infestation of *Cuscuta* spp., which compromised the vegetation cover and prevented the survey.

Table 5. Cover percentage of each plant family in the mixed grass plots, divided for treatment (D2 and D4, without and with compost respectively), and replicate (I, II, III).

Family *(Genus)*	**D2**			**D4**		
	I	II	III	I	II	III
Leguminosae % *(Trifolium)*	40.3	65.4	75.3	87.6	77.5	-
Gramineae % *(Lolium)*	59.7	34.6	24.7	12.4	22.5	-

In terms of biomass production, higher values were observed in the plots with sorghum compared to those with grass, and in plots amended with compost compared to those without (Figure 5). The effect of compost on vegetation surface density was more evident in the sorghum plots, which increased from 0.69 kg/m^2 (D1) to 1.67 kg/m^2 (D3), whereas the effect of vegetation type was more pronounced in the compost-amended plots. However, none of these differences were statistically significant.
No data were collected for spontaneous vegetation in the untreated plots (D0).

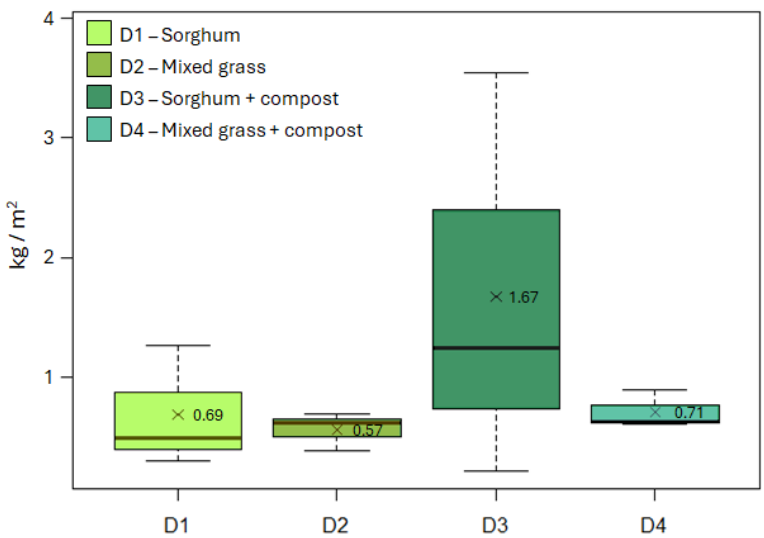


Figure 5. Boxplot and mean values of vegetation surface density in the experimental plots (D1, D2, D3, D4).

DISCUSSION

Short-term effects of Desealing and RA addition

At the beginning of the experiment, no spatially relevant differences were detected within the study area that could influence the subsequent evolution of soil characteristics and affect the comparisons between plots and treatments.

During the first three months after desealing, soil properties remained largely unchanged, and no significant differences were detected between untreated control plots and RA-treated plots. However, the latter exhibited a transient increase in pH, carbonates, organic carbon and total nitrogen. The early alkalinization can be attributed to the chemical composition of the aggregates, which mainly consists of concrete fragments rich in alkaline compounds, as discussed in Chapter 2. The observed increases in organic carbon and total nitrogen, on the other hand, may result from the incorporation of organic matter unintentionally collected from the soil surface and mixed with CDW during pavement removal [23]. This organic fraction remains incorporated into the RAs and is subsequently reintroduced into the soil of the experimental plots when the aggregates are applied on the soil surface. This contribution is likely more significant in degraded pavements, where organic matter can accumulate not only on the surface but also within and beneath the pavement, or in cases where the fertile topsoil was not completely removed before sealing.

Analysis of bacterial communities indicated that both time after desealing and addition of RAs influenced bacterial community structure, with untreated plots maintaining a distinct composition compared to RA-amended plots. The strongest temporal changes occurred in comparison to time zero, reflecting early microbial responses to soil disturbance and environmental variations. As the pH is one of the main drivers of bacterial community composition in soils [24], these changes are likely related to the temporary alkalinization caused by the incorporation of concrete based RAs, that may have favored the proliferation of taxa adapted to more basic conditions. Other factors may also have contributed to these short-term changes, including the introduction of microorganisms originally associated with the RAs, as well as the modification of pedoclimatic conditions such as soil temperature, moisture, and aeration following desealing and RA addition (as described in Chapter 2).

Nevertheless, diversity indices (Shannon, Gini, ASV richness, and ChaoB) remained largely stable, suggesting that the soil desealing and RA addition could qualitatively alter the microbial community structure without substantially affecting overall diversity in the short term.

Experimental treatments

Soil properties

The second monitoring period, designed to assess the influence of different treatments on the temporal evolution of soil properties, revealed no significant changes over time in either the experimental or untreated plots, with the only exception of a seasonal increase in organic carbon and total nitrogen during spring and summer. This result is not surprising, given the slow rate of pedological processes and the limited external inputs, which make unlikely to observe measurable changes in soil chemical properties within a year.

Interestingly, the observed increases in SOC and total N in the experimental setup in July 2024 were primarily associated with vegetation type rather than compost addition, since the increases were significant only in mixed grass plots regardless of compost presence. In particular, the elevated nitrogen levels can be attributed to the presence of legumes, which comprised approximately 50% of the seed mixture and 60-82% of the species observed in floristic surveys, potentially enhancing soil nitrogen availability through biological fixation of atmospheric nitrogen (N_2), directly contributing to the total nitrogen content in the soil [25]. However, the increases observed in April are likely incidental, reflecting pre-existing soil heterogeneity or the accidental inclusion of organic matter during sampling, since the experimental treatments had been applied only two weeks earlier and vegetation had not yet established.

The CN ratio remained relatively stable over time and across treatments, with the only significant deviations corresponding to the temporary increases in organic matter observed in April. Overall, its mean values indicate good-quality organic matter in the untreated plots (9.9), whereas slightly lower values were observed in the experimental plots (ranging from 8 to 9, with a peak of 9.3 in July). These lower ratios suggest that organic matter in the experimental plots may have a relatively faster mineralization rate, favoring the release of nitrogen into the soil rather than humus accumulation. This faster mineralization may be driven by multiple factors such as pedoclimatic conditions, microbial community composition and activity, and vegetation diversity and type [26]. However, the specific influence of vegetation in this study is difficult to assess, since the spontaneous vegetation in the control plots was not monitored.

Another significant difference was observed in soil pH, which increased in Sorghum plot in July compared to time zero. This trend may reflect differences in root activity and rhizosphere processes associated with the two vegetation type, particularly in relation to root exudate composition [27], but it may also be partially explained by initial variations in soil pH among the experimental plots, as the mean pH of mixed grass soils was already slightly higher than that of Sorghum (7.1 and 6.9, respectively).

Bacterial communities

The redundancy analysis showed that both time and experimental treatments influenced bacterial community composition, although their combined effect accounted for only a negligible fraction of total variance (<10%).

Despite temporal variations, untreated plots maintained a distinct community compared to experimental plots with RAs, suggesting that interventions such as vegetation establishment and compost addition can modify bacterial community composition. However, these changes were relatively minor compared to natural seasonal dynamics. Indeed, the clustering of samples and the significant differences between sampling times suggested that seasonal factors, including climate variations and resource inputs such as root exudates and organic matter, played a major role in shaping bacterial community composition over medium timescales (one year) [7]. These results are consistent with previous studies showing that microbial community dynamics in temperate soils are strongly regulated by fluctuations in temperature, soil moisture, and plant phenology, which regulate carbon inputs and nutrient turnover [28,29].

Diversity indices did not differ among treatments but changed significantly over time, with ASV richness and ChaoB increasing toward the end of the monitoring period. This indicates a progressive colonization of the desealed soil by a larger number of taxa, consistent with the combined effects of favorable microhabitat formation, the reactivation of biological exchanges between soil and atmosphere after desealing, and the gradual inputs from root exudates, plant residues, or decomposing organic matter. In the long term, this trend may enhance soil functionality by supporting more complex and stable microbial communities.

Vegetation growth

At the beginning of the monitoring period, germination appeared slower in the compost-amended plots compared to the others, likely due to the higher porosity and reduced water retention of the freshly applied compost, compared to the RA-only plots. This effect was probably accentuated by the manual application and incorporation of compost into the surface of RA layer, which may have resulted in lower compaction and uneven moisture distribution.

Subsequently, as expected, compost addition promoted vegetation establishment and growth, as indicated by the greater color intensity and height of Sorghum plants (Figure S5). Dry biomass measurements confirmed this trend, although the differences between compost and non-compost plots were not statistically significant, due to the high variability detected among the replicates. Compost application also appeared to affect species composition within the mixed grass plots, increasing the relative abundance of Leguminosae (82%) compared to Gramineae. This trend is consistent with previous evidence suggesting that compost can promote soil conditions that are favorable to nodule formation and nitrogen fixation in legumes, thereby improving their competitiveness and growth [30].

The variability observed in vegetation growth, both within plots and among replicates of the same treatment, was likely driven by surface irregularities in the RA layer, uneven seed or compost distribution, and small-scale microenvironmental differences such as light exposure and moisture availability, which commonly influence early plant establishment.

CONCLUSION

The addition of RAs caused an immediate but temporary increase in soil pH, which subsequently returned to initial values, and that influenced the structure of the bacterial community in the early stages after their application. Despite these initial effects, RA addition did not limit vegetation establishment or growth, and temporal dynamics in bacterial community structure were largely driven by seasonal factors rather than by the experimental treatments.

Overall, the experimental treatments did not produce significant variations in soil chemical properties or microbial community, with only minor and transient changes in nitrogen content and pH. Minor changes were attributed to vegetation type, while most temporal fluctuations were associated with seasonal variations in pedoclimatic conditions, such as temperature, moisture, and organic inputs.

In conclusion, the study confirm that desealing can promote the gradual recovery of soil functions and ecosystem processes over the medium to long term, by creating favorable conditions for vegetation establishment and microbial activity, even though early changes in soil properties and bacterial diversity were limited. It also confirm that RA application allow vegetation establishment, that was successful across al the experimental treatments, demonstrating its compatibility with soil restoration and revegetation processes.

These findings highlight the potential of combining desealing with in situ RA reuse for urban soil recovery, while contributing to the reduction of both economic and environmental costs associated with CDW disposal. At the same time, they also emphasize the need of long-term studies to fully assess the benefits of such interventions and optimize these practices for enhancing biodiversity and supporting resilient green infrastructures.

Bibliography

1. Food and Agriculture Organization of the United Nations *Status of the World's Soil Resources (SWSR) – Main Report*; 2015;
2. Food and Agriculture Organization of the United Nations; Intergovernmental Technical Panel on Soils; Global Soil Biodiversity Initiative; Secretariat of the Convention on Biological Diversity; European Commission *The State of Knowledge of Soil Biodiversity – Status, Challenges and Potentialities*; 2020;
3. Ferreira, C.S.S.; Walsh, R.P.D.; Ferreira, A.J.D. Degradation in Urban Areas. *Curr Opin Environ Sci Health* 2018, *5*, 19–25, doi:10.1016/j.coesh.2018.04.001.
4. Stolte, J.; Tesfai, M.; Øygarden, L.; Kværnø, S.; Keizer, J.; Verheijen, F.; Panagos, P.; Ballabio, C.; Hessel, R. *Soil Threats in Europe – Status, Methods, Drivers and Effects on Ecosystem Services*; Publications Office of the European Union, 2015;
5. JRC Publications Repository - Reports of the Technical Working Groups Established under the Thematic Strategy for Soil Protection. Vol. VI: Research, Sealing and Cross-Cutting Issues. Available online: https://publications.jrc.ec.europa.eu/repository/handle/JRC28869 (accessed on 30 October 2025).
6. Desrousseaux, M.; Schmitt, B.; Billet, P.; Béchet, B.; Le Bissonnais, Y.; Ruas, A. Artificialised Land and Land Take: What Policies Will Limit Its Expansion and/or Reduce Its Impacts? In; 2019; pp. 149–165.
7. Bardgett, R.D.; van der Putten, W.H. Belowground Biodiversity and Ecosystem Functioning. *Nature* 2014, *515*, 505–511, doi:10.1038/nature13855.
8. Fierer, N. Embracing the Unknown: Disentangling the Complexities of the Soil Microbiome. *Nat Rev Microbiol* 2017, *15*, 579–590, doi:10.1038/nrmicro.2017.87.
9. Renella, G. Evolution of Physico-Chemical Properties, Microbial Biomass and Microbial Activity of an Urban Soil after de-Sealing. *Agriculture (Switzerland)* 2020, *10*, 1–11, doi:10.3390/agriculture10120596.
10. Maienza, A.; Ungaro, F.; Baronti, S.; Colzi, I.; Giagnoni, L.; Gonnelli, C.; Renella, G.; Ugolini, F.; Calzolari, C. Biological Restoration of Urban Soils after De-Sealing Interventions. *Agriculture (Switzerland)* 2021, *11*, 1–12, doi:10.3390/agriculture11030190.
11. European Commission and Directorate-General for Environment *Guidelines on Best Practice to Limit, Mitigate or Compensate Soil Sealing*; Publications Office, 2012;
12. Caselli, B.; Ceci, M.; De Noia, I.; Garda, E.; Zazzi, M. Towards the Integration of Soil Desealing in the Urban Areas' Transformation Processes. In; 2024; pp. 286–298.
13. Adobati, F.; Garda, E. Soil Releasing as Key to Rethink Water Spaces in Urban Planning. *City, Territory and Architecture* 2020, *7*, 9, doi:10.1186/s40410-020-00117-8.
14. Vieillard, C.; Vidal-Beaudet, L.; Dagois, R.; Lothode, M.; Vadepied, F.; Gontier, M.; Schwartz, C.; Ouvrard, S. Impacts of Soil De-Sealing Practices on Urban Land-Uses, Soil Functions and Ecosystem Services in French Cities. *Geoderma Regional* 2024, *38*, e00854, doi:10.1016/j.geodrs.2024.e00854.
15. Aimar, F. Limiting Soil Sealing and Depaving: Local Actions for Regenerating Public Spaces to Build Green Infrastructures. In; 2023; pp. 127–137.
16. European Commission; Directorate-General for Internal Market, I.E. and Sme.; Oberender, A.; Fruergaard Astrup, T.; Frydkjær Witte, S.; Camboni, M.; Chiabrando, F.; Hayleck, M.; Akelytė, R. *EU Construction & Demolition Waste Management Protocol Including Guidelines for Pre-Demolition and Pre-Renovation Audits of Construction Works – Updated Edition 2024*; Publications Office of the European Union, 2024;
17. Fabbri, D.; Pizzol, R.; Calza, P.; Malandrino, M.; Gaggero, E.; Padoan, E.; Ajmone-Marsan, F. Constructed Technosols: A Strategy toward a Circular Economy. *Applied Sciences (Switzerland)* 2021, *11*, doi:10.3390/app11083432.
18. Mastrolando, G.; Giagnoni, L.; Maienza, A.; Fantoni, G.; Rompato, B.; Argenti, G.; Selvi, F.; Fiore, B.; Certini, G. Use of Waste Materials as Feasible Strategy for Urban De-Sealed Soil Reclamation: Evidence from a Restoration Case Study in Prato, Italy. In Proceedings of the 13th International Symposium of the International Union of Soil Sciences on Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA 13); 2025; pp. 158–159.
19. Coull, M.; Butler, B.; Hough, R.; Beesley, L. A Geochemical and Agronomic Evaluation of Technosols Made from Construction and Demolition Fines Mixed with Green Waste Compost. *Agronomy* 2021, *11*, doi:10.3390/agronomy11040649.

20. COMMISSION DECISION of 18 December 2014 Amending Decision 2000/532/EC on the List of Waste Pursuant to Directive 2008/98/EC of the European Parliament and of the Council . *Official Journal of the European Union* 2014.
21. D.M. n. 186 - Min. Dell'Ambiente e Della Tutela Del Territorio Decreto 05/04/06 - Tab. p. m; Regolamento UE N. 1357/2014 Della Commissione Del 18 Dicembre 2014.
22. Gandolfi, I.; Canedoli, C.; Rosatelli, A.; Covino, S.; Cappelletti, D.; Sebastiani, B.; Tatangelo, V.; Corengia, D.; Pittino, F.; Padoa-Schioppa, E.; et al. Microbiomes of Urban Trees: Unveiling Contributions to Atmospheric Pollution Mitigation. *Front Microbiol* 2024, *15*, doi:10.3389/fmicb.2024.1470376.
23. Page, J.L.; Winston, R.J.; Hunt III, W.F. Soils beneath Suspended Pavements: An Opportunity for Stormwater Control and Treatment. *Ecol Eng* 2015, *82*, 40–48, doi:10.1016/j.ecoleng.2015.04.060.
24. Fierer, N.; Jackson, R.B. The Diversity and Biogeography of Soil Bacterial Communities. *Proceedings of the National Academy of Sciences* 2006, *103*, 626–631, doi:10.1073/pnas.0507535103.
25. Kebede, E. Contribution, Utilization, and Improvement of Legumes-Driven Biological Nitrogen Fixation in Agricultural Systems. *Front Sustain Food Syst* 2021, *5*, doi:10.3389/fsufs.2021.767998.
26. Lama, S.; Velescu, A.; Leimer, S.; Weigelt, A.; Chen, H.; Eisenhauer, N.; Scheu, S.; Oelmann, Y.; Wilcke, W. Plant Diversity Influenced Gross Nitrogen Mineralization, Microbial Ammonium Consumption and Gross Inorganic N Immobilization in a Grassland Experiment. *Oecologia* 2020, *193*, 731–748, doi:10.1007/s00442-020-04717-6.
27. Vives-Peris, V.; de Ollas, C.; Gómez-Cadenas, A.; Pérez-Clemente, R.M. Root Exudates: From Plant to Rhizosphere and Beyond. *Plant Cell Rep* 2020, *39*, 3–17, doi:10.1007/s00299-019-02447-5.
28. Lauber, C.L.; Ramirez, K.S.; Aanderud, Z.; Lennon, J.; Fierer, N. Temporal Variability in Soil Microbial Communities across Land-Use Types. *ISME J* 2013, *7*, 1641–1650, doi:10.1038/ismej.2013.50.
29. Fierer, N.; Strickland, M.S.; Liptzin, D.; Bradford, M.A.; Cleveland, C.C. Global Patterns in Belowground Communities. *Ecol Lett* 2009, *12*, 1238–1249, doi:10.1111/j.1461-0248.2009.01360.x.
30. Tortosa, G.; Mesa, S.; Delgado, M.J.; Amaya-Gómez, C. V. “Alperujo” Compost Improves Nodulation and Symbiotic Nitrogen Fixation of Soybean Inoculated with Bradyrhizobium Diazoefficiens. *Nitrogen* 2023, *4*, 223–230, doi:10.3390/nitrogen4020015.

Supplementary materials

List of Figures

Figure S1. Experimental site with concrete pavement prior to desealing operations (September 2023).

Figure S2. Desealed soil surface after desealing and before to RA addition (October 2023).

Figure S3. Experimental site after treatments establishment (April 2024).

Figure S4. Germination of *Lolium* and *Trifolium* species on the RA layer without compost addition.

Figure S5. Differences in sorghum growth according to compost addition (July 2025).

List of Tables

Table S1. Mean values and standard errors (SE) of soil properties divided by treatments (D0, D1, D2, D3, D4) and sampling times: October (t0) 2023 and April (t4), July (t5) and October (t6) 2024. Different letters in the "group" column indicate significant differences ($p < 0.05$): (a) among sampling times within each treatment and (b) among treatments within each sampling time.

(a)

Plot	Time	pH			$CaCO_3$ (g/kg)			SOC (g/kg)			N (g/kg)			CN		
		Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.
D0	t0	7.10	0.12	a	3.50	2.02	a	0.75	0.02	a	0.078	0.001	b	9.63	0.32	a
	t4	7.35	0.09	a	1.50	0.87	a	0.68	0.01	a	0.072	0.002	ab	9.48	0.14	a
	t5	7.35	0.14	a	6.00	1.15	a	0.52	0.01	a	0.051	0.003	a	10.43	0.40	a
	t6	7.05	0.09	a	0.50	0.29	a	0.73	0.02	a	0.073	0.001	ab	10.05	0.11	a
D1	t0	6.97	0.03	a	1.00	0.00	a	0.61	0.06	a	0.075	0.007	a	8.12	0.29	a
	t4	7.00	0.06	a	1.67	1.20	a	0.63	0.01	a	0.070	0.003	a	9.04	0.26	a
	t5	7.47	0.24	a	4.33	2.40	a	0.60	0.05	a	0.062	0.006	a	9.68	0.40	a
	t6	7.27	0.09	a	1.33	0.67	a	0.69	0.04	a	0.080	0.005	a	8.66	0.15	a
D2	t0	6.83	0.22	a	6.67	6.17	a	0.66	0.05	a	0.084	0.011	a	7.93	0.45	a
	t4	6.97	0.26	a	1.00	1.00	a	0.73	0.10	a	0.084	0.009	a	8.70	0.56	a
	t5	7.03	0.18	a	7.67	7.17	a	0.71	0.05	a	0.080	0.006	a	8.93	0.82	a
	t6	7.13	0.18	a	4.33	2.33	a	0.71	0.07	a	0.083	0.005	a	8.55	0.35	a
D3	t0	6.80	0.26	a	1.67	1.67	a	0.55	0.06	a	0.071	0.009	a	7.73	0.14	a
	t4	6.87	0.09	a	0.00	0.00	a	0.53	0.12	a	0.071	0.015	a	7.50	0.20	a
	t5	7.30	0.17	a	0.33	0.33	a	0.66	0.06	a	0.074	0.005	a	9.07	1.40	a
	t6	6.97	0.35	a	0.33	0.33	a	0.61	0.07	a	0.077	0.011	a	7.97	0.40	a
D4	t0	7.10	0.06	a	3.67	2.33	a	0.67	0.09	a	0.072	0.005	a	9.37	1.21	a
	t4	6.83	0.33	a	2.67	1.76	a	0.82	0.10	a	0.095	0.007	b	8.56	0.44	a
	t5	7.27	0.22	a	4.67	3.28	a	0.82	0.09	a	0.086	0.003	ab	9.54	1.32	a
	t6	7.23	0.27	a	2.33	1.86	a	0.76	0.05	a	0.086	0.005	ab	8.77	0.15	a

(b)

Time	Plot	pH			CaCO3 (g/kg)			SOC (g/kg)			N (g/kg)			CN		
		Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.	Mean	SE	gr.
t0	D0	7.10	0.12	a	3.50	2.02	a	0.75	0.02	a	0.078	0.001	a	9.63	0.32	a
	D1	6.97	0.03	a	1.00	0.00	a	0.61	0.06	a	0.075	0.007	a	8.12	0.29	a
	D2	6.83	0.22	a	6.67	6.17	a	0.66	0.05	a	0.084	0.011	a	7.93	0.45	a
	D3	6.80	0.26	a	1.67	1.67	a	0.55	0.06	a	0.071	0.009	a	7.73	0.14	a
	D4	7.10	0.06	a	3.67	2.33	a	0.67	0.09	a	0.072	0.005	a	9.37	1.21	a
t4	D0	7.35	0.09	a	1.50	0.87	a	0.68	0.01	ab	0.072	0.002	ab	9.48	0.14	b
	D1	7.00	0.06	a	1.67	1.20	a	0.63	0.01	ab	0.070	0.003	a	9.04	0.26	ab
	D2	6.97	0.26	a	1.00	1.00	a	0.73	0.10	ab	0.084	0.009	ab	8.70	0.56	ab
	D3	6.87	0.09	a	0.00	0.00	a	0.53	0.12	a	0.071	0.015	a	7.50	0.20	a
	D4	6.83	0.33	a	2.67	1.76	a	0.82	0.10	b	0.095	0.007	b	8.56	0.44	ab
t5	D0	7.35	0.14	a	6.00	1.15	a	0.52	0.01	a	0.051	0.003	a	10.43	0.40	a
	D1	7.47	0.24	a	4.33	2.40	a	0.60	0.05	ab	0.062	0.006	ab	9.68	0.40	a
	D2	7.03	0.18	a	7.67	7.17	a	0.71	0.05	ab	0.080	0.006	b	8.93	0.82	a
	D3	7.30	0.17	a	0.33	0.33	a	0.66	0.06	ab	0.074	0.005	ab	9.07	1.40	a
	D4	7.27	0.22	a	4.67	3.28	a	0.82	0.09	b	0.086	0.003	b	9.54	1.32	a
t6	D0	7.05	0.09	a	0.50	0.29	a	0.73	0.02	a	0.073	0.001	a	10.05	0.11	a
	D1	7.27	0.09	a	1.33	0.67	a	0.69	0.04	a	0.080	0.005	a	8.66	0.15	a
	D2	7.13	0.18	a	4.33	2.33	a	0.71	0.07	a	0.083	0.005	a	8.55	0.35	a
	D3	6.97	0.35	a	0.33	0.33	a	0.61	0.07	a	0.077	0.011	a	7.97	0.40	a
	D4	7.23	0.27	a	2.33	1.86	a	0.76	0.05	a	0.086	0.005	a	8.77	0.15	a

CHAPTER 4

Impacts of Recycled Concrete Aggregates and Reclaimed Asphalt Pavement on soil microbial activities and physicochemical properties: an indoor-mesocosm experiment

Gaia Mascetti[a], Chiara Ferré[a], Isabella Gandolfi[a], Susana Loureiro[b], Sara Peixoto[b]

[a] DISAT-Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano Bicocca, Milano, Italia; [b] CESAM- Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal.

In preparation

Abstract

The reuse of construction and demolition waste as soil constituent material offers promising opportunities for circular economy and urban soil restoration, yet the environmental effects of recycled aggregates in soil are still largely unknown. This chapter presents an indoor mesocosm experiment designed to assess the impacts of recycled concrete aggregates (RCA) and reclaimed asphalt pavement (RAP) on soil physicochemical properties, microbial activity, and phytotoxicity. Standard soils (Lufa 2.1 and Lufa 2.2) were amended with a fine fraction of recycled aggregates (<1 cm) under different moisture regimes. Changes in water retention, compaction, pH, nutrient availability, enzymatic activities (DHA, phosphatase, arylsulfatase and urease), microbial basal respiration, and early-stage plant development were evaluated.

The results revealed that, while aggregate addition generally improved soil physical properties such as drainage and compaction, chemical and microbial responses were strongly material-specific. RCA induced the largest increases in soil pH and electrical conductivity, accompanied by significant reductions in nutrient availability and enzymatic activities. In contrast, RAP caused more moderate alterations, but still affected soil chemical properties, with indirect consequences for microbial activities. Phytotoxicity assays indicated reduced germination and early plant growth at the tested RCA concentrations, while RAP showed more moderate effects and, under certain conditions, supported plant development.

Overall, the results underscore the need for careful assessment prior to the use of recycled aggregates in soil restoration practices, as their effects are strongly dependent on aggregate composition, initial soil properties, and environmental conditions.

INTRODUCTION

Construction and Demolition Waste (CDW) generated by construction and demolition activities, with the exception of uncontaminated soil [1], represents 40% of the total waste stream in Europe, with its volume expected to double between 2020 and 2050 [2,3]. Recovery and utilization of CDW are relatively high in European Union (EU) countries, with more than 70% of the mineral fraction recovered in 2018 [3]. However, despite EU initiatives promoting CDW reuse and recycling, these practices are not sufficiently implemented, and in many member states, most CDW is still managed through backfilling [1], for example, for landscaping purposes [3]. In the last decades, emerging technologies and solutions have been studied and developed for the management of concrete waste, which is a predominant fraction in the CDW [3]. Following appropriate end-of-waste processes (*e.g.* cleaning, crushing and sieving), concrete waste can be converted into recycled concrete aggregates (RCA) and used in the production of new concrete. However, according to the EU, the fine fraction (0-4 mm), which constitutes around 40% of the crushed material, cannot be used for this purpose [3]. Therefore, this fraction is currently used primarily for road construction and backfilling, selecting the best recovery solution based on its properties, technical feasibility, environmental and economic costs associated with the process (including transportation), and its outputs [3].

A growing source of concrete CDW is currently represented by the restoration and urban greening initiatives [3], such as desealing interventions, which involve the removal and demolition of paved surfaces. However, these activities also contribute to the accumulation of asphalt waste, generated from the milling (milled asphalt) or the full-depth removal of asphalt pavement, such as roads and parking areas [4–6]. In recent years, reclaimed asphalt pavement (RAP), the recycled granular material from the milled or ripped asphalt,– has been used for recycled asphalt mixtures or as aggregate in base and subbase construction [5,7]. In Europe, seventeen countries produce approximately 36 million tons of RAP a year [8]. The European standard EN 13108-8 specifies guidelines for its use in asphalt mixtures, emphasizing careful assessment of material source, homogeneity, and requiring several numbers of testing samples [8].

In recent years, several studies have investigated alternative and sustainable uses of demolition waste. Among them, an increasing number of studies are focusing on the potential reuse of CDW and RAP as components of constructed or engineered soils for urban restoration and greening, especially after desealing interventions. Field and laboratory studies have demonstrated the potential of demolition waste and milled asphalt as growth substrates for various plant species [4,5,9,10], and highlighted their capacity to enhance soil macroporosity, thereby improving air and water flow [5,10]. These results suggested that they can be used as structural materials for preventing soil compaction in urban areas [11]. Additional research has investigated the reactivity of demolition waste in soil [12], as well as its toxicity effects on leaching and on microbial activity, structure, and community composition [5,10]. Other studies have investigated the ecotoxicological impact of CDW and new concrete containing recycled materials. However, they focused on water ecosystems, performing leaching tests followed by chemical and ecotoxicological risk assessment, using aquatic organisms such as freshwater algae, crustaceans and marine bacteria [13–15].

Thus, while most studies focused on the effects of recycled materials on leaching and soil physicochemical properties, the combined environmental and biological implications of incorporating recycled aggregates into soil remain limited. Further studies are needed to understand their complex impact on soil ecosystems, investigating the functional responses of soil microbial communities and the ecotoxicological effects on soil biota. Trying to address this knowledge gap, the objectives of this research are to investigate the effects of RCA and RAP (i) on soil microbial communities' function, (ii) on physicochemical soil properties, (iii) under different climatic condition scenarios, and iv) on plant species germination and growth. For this purpose, an indoor-mesocosm experiment was conducted using mixtures of aggregates and standard soils, to evaluate biological endpoints (*i.e.*, enzymatic activities, microbial basal respiration and phytotoxicity, assessed through germination and plant growth) and soil physicochemical properties (*i.e.*, compaction, percolation, pH, electrical conductivity and soil nutrient content) under simulated standard and flooding conditions.

MATERIAL AND METHODS

Lufa Soil and Recycled Aggregates

The test soils selected were Lufa 2.2 and Lufa 2.1 (Speyer, Germany), which are natural and reference soils in soil ecotoxicology, allowing for reproducibility and comparability of results [16]. The two selected soils primarily differ in terms of acidity, organic matter content, texture, and water-holding capacity (WHC). Lufa 2.2 is a sandy loam soil with a pH of 5.5 ± 0.1 (in $CaCl_2$ 0.01 M), organic carbon content of 1.66 ± 0.60 %, total nitrogen content of 0.19 ± 0.06 %, cation exchange capacity of 8.5 ± 1.8 meq/100 g, maximum water holding capacity of 56.0 %, and a bulk density of 1.23 ± 0.94 g/cm^3. Lufa 2.1 is a sand soil with a pH of 4.69 ± 0.29 (in $CaCl_2$ 0.01 M), organic carbon content of 0.7 ± 0.26 %, total nitrogen content of 0.07 ± 0.02

%, cation exchange capacity of 3.68 ± 0.73 meq/100 g, maximum water holding capacity of 29.1 %, and a bulk density of 1.46 ± 0.04 g/cm^3.

Recycled concrete aggregates (RCA) and reclaimed asphalt pavement (RAP) were provided by Sereca Sas and Edilnapoli Srl, respectively. The characteristics of both materials are provided in Supplementary Materials (Appendix S1 and S2).

Experimental design

Indoor-mesocosms experiments were carried out by mixing RCA or RAP with Lufa 2.2 or 2.1 to perform three treatments for each standard soil (Figure 1): non-mixed soil as control (CT), soil mixed with asphalt (MixA) and soil mixed with concrete (MixC). Soils and recycled materials were mixed manually in 1:1 volumetric ratio to obtain homogeneous mixtures, brought to 50% WHC with ultrapure water, and transferred into PVC columns (high 20 cm and with 11 cm of diameter), closed at the bottom with a 1 mm PVP nylon mesh.

The experiments were performed under two moisture regimes: standard moisture (50% of WHC) and flooding conditions. For flooding conditions, two rainfall events were simulated at day 0 and day 14 by slowly adding to each column the exact volume of water to reach 110% of WHC. Starting from day 1, a volume of 5 ml and 20 ml of artificial rainwater [NaCl (0.01 mM), $(NH_4)_4 SO_4{\cdot}H_2O$ (0.0053 mM), $NaNO_3$ (0.0059 mM), and $CaCl_2{\cdot}H_2O$ (0.0039 mM); pH= 5.1] [29] was daily added to the columns to maintain, respectively, standard and flooding conditions.

Six columns were prepared for each treatment and moisture regimes, and the experiments were run for 28 days in controlled conditions [soil temperature 15°C; air temperature 20 ± 2°C; photoperiod 16 h (light):8 h (dark)]. At each sampling time (day 14 and day 28), three columns (three replicates) of each treatment were removed to collect representative soil samples (Figure 1). Regarding time zero, soil samples were collected immediately before building the columns. All the soil samples were: 1) stored at 4°C for the assessment of microbial activities, and 2) dried for the chemical analyses. Percolation water was collected during soil sampling times (day 14 and 28) and after 24 h (day 1 and/or 15) from each rainfall event.

During the experiments, soil moisture was monitored by periodically weighing the columns, while soil compaction was estimated by measuring the distance from the top of the PVC column to the soil surface (in cm). Funnels and cups were placed underneath the columns to allow the collection of leachate and estimate water percolation. Throughout this section, the term “soil” will be used to indicate both soil and mixture samples.

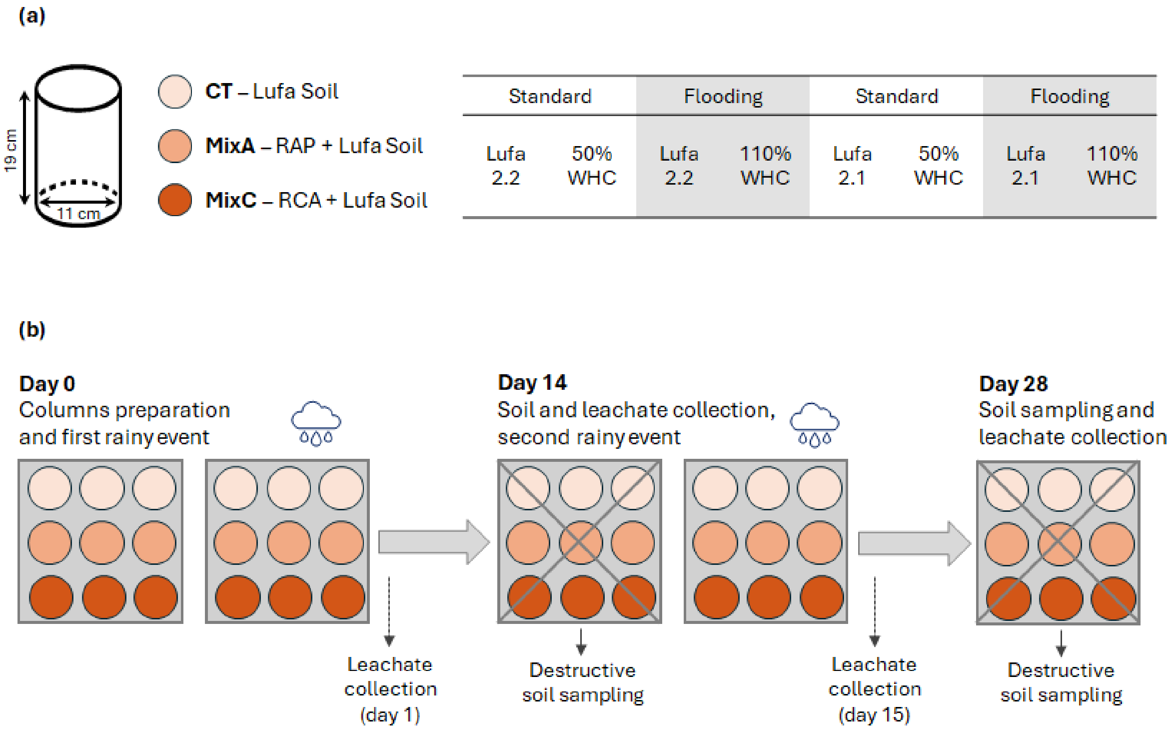


Figure 1. Schematic representation of the **(a)** experimental design and **(b)** temporal procedure of the indoor-mesocosm experiment. The experiment was performed with different combinations of Lufa soil (2.2 or 2.1) and moisture regimes: standard and flooding conditions, with 50% or 100% of the water-holding capacity (WHC), respectively. The rainy events and leachate collection were performed only for the experiment under flooding conditions.

Physical and Chemical Analyses

Immediately after sampling (days 0, 14 and 28), 5 grams of soil were mixed and shaken with 25 mL of ultra-pure water, and pH and electrical conductivity (EC) were measured, according to the standard ISO standard protocol 10390 [17]. About 10 grams of soil were oven-dried (60°C) and weighed to assess the moisture content.

At the last sampling time (day 28), a further amount of soil was air-dried at room temperature and sieved at 2 mm for standard laboratory analyses. The reaction of soil fine fraction was determined by pH in water (pH; soil to water ratio of 1:2.5), and total carbonates were determined on alkaline samples with the Dietrich-Frühling calcimetric method. Organic carbon (SOC) and total nitrogen contents were determined by dry combustion of soil sieved at 0.5 mm using a Flash EA 1112 NC-Soil elemental analyzer (Thermo Fisher Scientific CN, Pittsburgh, USA). Available, organic and total phosphorus were also determined according to the Italian regulation D.M. of 13/09/1999 [18].

Assessment of Soil Microbial Activities

Enzymatic activities

Dehydrogenase (DHA), acid and alkaline phosphatase (ACP and ALP), urease (UR) and arylsulfatase (ARS) activities were determined to the methods described by Dick et al. [19], with some modifications.

The DHA activity was evaluated using soil samples (3 g) in a 3% triphenyl tetrazolium chloride aqueous solution (TTC; Sigma-Aldrich, 98%). After 24 h of incubation at 37°C, the triphenyl formazan (TPF, Sigma-Aldrich, 98%) produced was extracted with methanol (Sigma-Aldrich, 99%), and the samples were centrifuged for 15 min at 3000 rpm. The absorbance was measured at a wavelength of 485 nm.

The ACP, ALP and ARS activities were measured using specific substrate solutions: *p-nitrophenyl* phosphate (PNP 0.5 M; Sigma-Aldrich, 99%) and *p-nitrophenyl* sulphate (PNS 0.05 M; Acros Organics, 99%), respectively. Soil samples (0.5 g) were incubated 1 h at 37°C with substrate solutions (PNP or PNS) and modified universal buffer (MUB) for phosphatases (pH=6.5 for ACP; pH=11 ALP) and acetate buffer for ARS (0.5 M; pH=5.8). The reaction was stopped with $CaCl_2$ (0.5 M) and NaOH (0.5 M), then samples were centrifuged (3000 rpm, 15 min), and the supernatant absorbance was measured at 410 nm.

For UR activity, soil samples (1 g) were incubated for 2h at 37°C with urea substrate solution (720 mM) and borate buffer (0.1 M, pH=10). The reaction was stopped with KCl solution, the samples were shaken for 30 minutes and centrifuged (3000 rpm, 15 min). The supernatant was treated with the appropriate reagents, allowed to react for 30 min at room temperature, and the absorbance was then read at 690 nm [20].

All enzymatic activities were quantified using 96-wells microplates, with four technical replicates for each sample, and a microplate spectrophotometer (Thermo Scientific Multiskan Spectrum, USA).

Microbial basal respiration

The microbial basal respiration was performed as described by FAO (2023) [21]. After being sieved at 2 mm, 20 g of soil from each soil treatment were placed in a 500 ml hermetic glass jar, in which an absorption bottle with 10 mL NaOH (1 M) solution was also hung, and incubated at 23°C for 9 days in dark conditions. At regular intervals (24, 48, 72, 144 and 216 hours), the NaOH bottle was removed from the jar to measure the amount of CO_2 adsorbed by adding 5 mL of $BaCl_2$ (0.5 M) and 3-4 drops of phenolphthalein indicator (0.1%) into the bottles and titrating with HCl (1 M). After measurement, 10 mL of NaOH was replaced. Empty jars were used as controls, and the difference in HCl consumed volume between the treatments and the control was used to calculate the amount of CO_2 produced by the microbial community. Finally, the metabolic quotient (qCO_2), the amount of CO_2 carbon consumed for kg of dry soil each hour (mg kg^{-1} h^{-1}), was calculated as with the following equation:

$$qCO2 = \frac{(V_B - V_S) \times N \times 6 \times 1000}{W \times h}$$

where: V_B and V_S are the volume of HCl consumed by the blank and the sample, respectively; N the HCl concentration (0.1 N); W the weight of the dry soil sample (g); h the hours of incubation; and 6 the conversion factor considering that 1 mL of NaOH is equivalent to 6 mg of CO_2 carbon.

The cumulative CO_2 consumed was calculated by summing the metabolic quotients across all time intervals. Negative values were assumed to indicate no respiration and were set to zero.

Phytotoxicity tests

The effect of RCA and RAP on early life stages of plants was evaluated on two different species, *Lolium perenne* and *Medicago sativa*, using polyvinylchloride transparent plates (21 × 15.5 × 0.8 cm) with two compartments (Phytotoxkit, MicroBioTest Inc., Gent, Belgium; standardized guideline ISO 18763, 2016) [22,23]. These species were selected as they are commonly used in phytotoxicity assays and represent two major functional and taxonomic plant groups, with *L. perenne* being a monocotyledonous grass (Poaceae) and *M. sativa* a dicotyledonous legume (Fabaceae).

The bottom compartment of each plate was filled with the soil collected at the last sampling time (28 days) of the mesocosm experiment: 100 – 120 g for each treatment. Five seeds for each plant species were positioned on the soil at equal distances from each other, close to the top of the bottom compartment (Figure S1). The plates were closed and placed vertically into a growth chamber at 23°C for 7 days, initially in dark conditions (until 24h after germination occurred), and then with a photoperiod of 16 h (light):8 h (dark). Since the first day, the number of germinated seeds was monitored daily, and at the end of the test (day 7), the plants were carefully removed to measure the root and shoot length (mm), fresh (mg) and dry weight (the plants were oven-dried at 60°C). The root to shoot ratio (R:S), Seed Germination (SG), Relative Seed Germination (RSG), Relative Root Growth (RRG) and Germination Index (GI) were then calculated as followed [24]:

$$R{:}S = \frac{Mean\ roots\ dry\ weight}{Mean\ shoots\ dry\ weight}$$

$$SG = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ tested}$$

$$RSG = \frac{Number\ of\ seeds\ germinated\ in\ the\ treatments}{Number\ of\ seeds\ germinated\ in\ the\ control}$$

$$RRG = \frac{Mean\ roots\ lenght\ in\ the\ treatments}{Mean\ roots\ lenght\ in\ the\ control}$$

$$GI = RSG \times RRG$$

In the results section, RSG and RRG indices are expressed as percentage and thresholds for indicating relevant reductions or increases were set at $\leq$ 80% and $\geq$ 120%, respectively. Conversely, GI values were interpreted as follows [25]: GI ≤ 0.5 (high negative impact); $0.5 <$ GI ≤ 0.8 (moderate negative impact); $0.8 <$ GI ≤ 1.2 (lack of relevant impact); $1.2 <$ GI ≤ 1.5 (moderate positive impact); GI > 1.5 (high positive impact).

Statistical elaboration

To evaluate the differences in soil properties, enzymatic activities and phytotoxicity effects among the treatments, one-way ANOVA analyses were performed using Sigma plot V.12.5 (SysStat software Inc., CA, USA). Homogeneity of variance and normality of distributions were verified using Levene's test and Shapiro-Wilk's test, respectively. When significant differences were found ($p<0.05$), a post-hoc analysis was performed using the Tukey test to obtain multiple comparisons among control soils, MixA and MixC. When the normality or homogeneity test failed even after variables transformation (acidic phosphatase, Lufa 2.1, standard moisture condition, day 28), a Kruskal-Wallis one-way analysis of variance on ranks was used.

RESULTS

Soil physical and chemical properties

Water Content, Compaction and Percolation

Under standard conditions, both Lufa 2.1 and Lufa 2.2 soils maintained the moisture content within the 45-55% of WHC for all sampling times and treatments (Table 1). The only exception was the MixC treatment with Lufa 2.1, which showed a significantly higher water content (66% of WHC) compared to CT on both days 14 and 28. Under flooding conditions, moisture content in Lufa 2.1 varied from 78% WHC in MixA, 87% in CT and 98% in MixC. In Lufa 2.2, values were generally lower, from 70% in MixA, 80% in MixC and 84% in CT. Statistically significant differences were always observed between CT and MixA (except for Lufa 2.1 on day 28), and between CT and MixC in Lufa 2.2 on day 28 (Table S1).

Under standard moisture conditions, soil compaction estimated at day 28 was similar across Lufa soils and treatments (mean height ≈ 13 cm; Table 1), with no significant differences among them, except for MixA towards CT Lufa 2.1, which showed mean heights of 13.1 ± 0.6 cm and 12.8 ± 0.8 cm, respectively. Under flooding conditions, Lufa 2.2 showed slightly higher compaction values (≈ +0.3 cm) and high variability among replicates compared to Lufa 2.1. As expected, soil compaction was higher under flooding conditions than under standard conditions, and significant differences were observed among all soil treatments in both Lufa 2.1 and 2.2: CT soil showed itself to be more compacted than MixC, and MixC was more compacted than MixA ($p<0.05$, Table S1).

Regarding percolation water (Table 2), distinct patterns were observed between the Lufa soil types: Lufa 2.1 exhibited lower volumes within the 24 hours after each rainy event but higher volumes over the subsequent two weeks (days 14–28). However, no significant differences in water loss were observed among the columns of the same Lufa at any sampling time, except between CT and MixA after 28 days in Lufa 2.2. Since the first rainfall, both Lufa soils showed higher percolation water pH in MixA and MixC than in CT, with values ranging from 7.3-8.3 for MixA and 7.7-8.7 in MixC. Overall, water pH increased over time in CT and MixA, while MixC showed higher values within the 24 h after each rainy event. The differences between the CT and the mixture with recycled materials were always statistically significant for each Lufa at every sampling time ($p<0.05$; Table S2), except for MixA *vs*. CT 24 h after the second rainy event. Overall, electrical conductivity decreased over time in all the treatments in both Lufa 2.2 and Lufa 2.1, with just a temporary increase in MixA and MixC on day 14.

Statistically significant differences were observed between CT and the mixtures with recycled materials for both Lufa types at all sampling times ($p<0.05$; Table S2), except for MixC vs. CT 24 h after the first rainy event with Lufa 2.2. MixA showed EC values higher than CT and MixC after the first rainy event with both Lufa; however, after the second event, the highest values were observed in MixC.

Table 1. Soil compaction and moisture values (mean ± sd) for each Lufa soil, moisture conditions and sampling time. Compaction data of MixA and MixC at day 14 are not measured (ND = Not Determined).

Parameter	Lufa	Moisture	Treatment	Day 14	Day 28
Compaction	**2.2**	Standard	CT	13.1 ± 0.5	13.1 ± 0.1
			MixA	ND	12.6 ± 0.1
			MixC	ND	13.5 ± 0.2
		Flooding	CT	11.7 ± 0.3	10.7 ± 0.4
			MixA	12.3 ± 0.2	12.2 ± 0.1
			MixC	12.3 ± 0.3	11.8 ± 0.3
	2.1	Standard	CT	13.1 ± 0.5	12.8 ± 0.8
			MixA	14.0 ± 0.1	13.1 ± 0.6
			MixC	13.8 ± 0.6	13.2 ± 0.3
		Flooding	CT	11.5 ± 0.3	11.0 ± 0.1
			MixA	13.1 ± 0.2	12.5 ± 0.1
			MixC	12.5 ± 0.3	12.1 ± 0.1
Moisture	**2.2**	Standard	CT	51.6 ± 0.4	48.7 ± 0.5
			MixA	49.9 ± 3.3	46.2 ± 1.7
			MixC	57.3 ± 4.4	55.5 ± 3.0
		Flooding	CT	86.4 ± 1.3	86.9 ± 1.5
			MixA	66.9 ± 7.4	73.1 ± 3.5
			MixC	81.1 ± 7.6	79.9 ± 1.0
	2.1	Standard	CT	49.7 ± 0.9	49.1 ± 0.5
			MixA	51.9 ± 3.3	47.7 ± 3.9
			MixC	65.6 ± 6.1	66.7 ± 4.4
		Flooding	CT	82.7 ± 1.4	84.9 ± 4.4
			MixA	78.0 ± 8.0	78.4 ± 3.8
			MixC	98.1 ± 3.9	97.6 ± 14.8

Table 2. Collected volume (ml), pH and conductivity of percolation water (mean ± sd), by Lufa type and sampling time. Sampling days 1 and 15 correspond to the 24 hours after the first and second rainfall events, respectively (Figure 1).

Parameter	Lufa	Treatment	First Rainy Event		Second Rainy Event	
			day 1	day 14	day 15	day 28
Collected volume (ml)	**2.2**	CT	100.5 ± 6.4	170.9 ± 11.3	164.0 ± 23.6	92.3 ± 2.9
		MixA	110.5 ± 14.0	165.0 ± 4.4	144.2 ± 18.6	62.5 ± 1.5
		MixC	126.1 ± 16.7	168.3 ± 13.4	150.1 ± 7.4	69.0 ± 16.8
	2.1	CT	66.0 ± 3.4	183.5 ± 3.4	100 ± 13.8	132.6 ± 14
		MixA	69.0 ± 12.7	180.7 ± 19.7	105 ± 7.2	118.4 ± 3.0
		MixC	74.2 ± 2.5	180.0 ± 9.4	102 ± 18.6	125.6 ± 12.1

pH	**2.2**	CT	6.2 ± 0.0	7.4 ± 0.0	7.8 ± 0.0	7.9 ± 0.0
		MixA	7.5 ± 0.1	8.0 ± 0.1	7.9 ± 0.1	8.3 ± 0.1
		MixC	8.0 ± 0.1	7.7 ± 0.2	8.6 ± 0.3	8.1 ± 0.1
	2.1	CT	5.5 ± 0.1	6.1 ± 0.1	6.9 ± 0.2	6.8 ± 0.1
		MixA	7.3 ± 0.3	8.2 ± 0.1	8.0 ± 0.1	8.2 ± 0.1
		MixC	8.7 ± 0.3	8.3 ± 0.1	8.1 ± 0.1	8.1 ± 0.1
EC (µS)	**2.2**	CT	861 ± 41	487 ± 40	286 ± 19	257 ± 18
		MixA	1708 ± 264	1948 ± 55	816 ± 191	955 ± 72
		MixC	1290 ± 249	1894 ± 214	1329 ± 113	1382 ± 139
	2.1	CT	819 ± 30	474 ± 24	167 ± 25	116 ± 1
		MixA	1777 ± 200	1827 ± 91	1039 ± 46	897 ± 51
		MixC	1524 ± 81	1678 ± 95	1376 ± 82	1235 ± 110

Soil pH and Conductivity

The pH differences among the soil treatments (CT *vs*. MixA; CT vs. MixC; MixA vs. MixC) were statistically significant regardless Lufa type, moisture regimes and sampling time (Table S3 and S4). At day 0, the soil pH of Lufa 2.2 and 2.1 (CT) were 6.3 and 5.4, respectively, but the addition of recycled materials immediately raise pH values to 7.6 (MixA) and 10.4-10.7 (MixC with Lufa 2.2 and Lufa 2.1, respectively) (Table S3). During the experiments, pH values in Lufa 2.2 control columns remain stable under both soil moisture conditions (Table 3). Compared to day 0, under standard soil moisture conditions, MixC showed a slight reduction in pH after 28 days (9.5), while under flooding conditions, both MixA and MixC showed a significant pH increase, reaching values of 8.4 and 11.0, respectively. In Lufa 2.1 soil, pH increased under flooding conditions (6.1), while in standard conditions it initially decreased (4.7) and returned to initial values (5.5). Compared to day 0, MixA didn't show relevant variations, while MixC showed a further increase of pH in flooding conditions (>11, Table 3). EC differences among the soil treatments were always statistically significant, regardless of Lufa type, moisture conditions and sampling time, except for MixA *vs*. MixC in Lufa 2.2 at day 0 (Table S3 and S4). The addition of recycled materials resulted in a significant increase in EC from day 0: from 58 µS to 182 µS (MixA) and 210 µS (MixC) with Lufa 2.2; and from 39 µS to 154 µS (MixA) and 293 µS (MixC) with Lufa 2.1. In Lufa 2.2, a decrease in EC was then observed in CT and MixA in both moisture regimes. In contrast, MixC showed a general increase, even though pH returned to initial values after 28 days under flooding conditions (Table 3). Regarding Lufa 2.1, a decrease in EC was observed in CT under flooding conditions, in MixA in both moisture conditions, and in MixC in standard conditions. In contrast, an initial increase in EC occurred in MixC under flooding conditions, which then returned to the original values after 28 days.

Table 3. Soil pH and electrical conductivity (EC) after 28 days (mean ± sd), grouped by Lufa type and moisture conditions.

Parameter	Lufa	Moisture	Treatment	Day 14	Day 28
pH	**2.2**	Standard	CT	6.33 ± 0.05	6.08 ± 0.01
			MixA	7.58 ± 0.02	7.82 ± 0.03
			MixC	10.49 ± 0.08	9.49 ± 0.03
		Flooding	CT	6.47 ± 0.05	6.23 ± 0.03
			MixA	7.57 ± 0.03	8.42 ± 0.07
			MixC	11.03 ± 0.01	10.97 ± 0.04
	2.1	Standard	CT	4.68 ± 0.03	5.54 ± 0.04
			MixA	7.96 ± 0.07	7.86 ± 0.03
			MixC	10.67 ± 0.30	10.60 ± 0.15
		Flooding	CT	5.92 ± 0.04	6.09 ± 0.06
			MixA	7.86 ± 0.02	7.77 ± 0.09
			MixC	11.32 ± 0.07	11.13 ± 0.08
EC (µS)	**2.2**	Standard	CT	51.1 ± 6.7	52.1 ± 6.3
			MixA	171.7 ± 13.1	161.4 ± 13.5
			MixC	302.7 ± 12.0	320.7 ± 49.5
		Flooding	CT	28.6 ± 2.5	25.6 ± 2.7
			MixA	102.7 ± 11.5	85 ± 4.2
			MixC	324.3 ± 7.3	213.7 ± 11.0
	2.1	Standard	CT	41.7 ± 2.2	43.2 ± 5.0
			MixA	117.4 ± 2.3	132.0 ± 0.8
			MixC	230.0 ± 15.4	259.0 ± 2.6
		Flooding	CT	21.5 ± 13.1	22.2 ± 3.5
			MixA	91.0 ± 11.3	104.9 ± 6.4
			MixC	330.7 ± 14.3	306.3 ± 17.4

Soil Nutrient Content

At day 0, Lufa 2.2 soil was characterized by medium-high values of organic carbon: 28.7 g/kg, and total nitrogen: 2.5 g/kg (Table 4). After 28 days, organic carbon significantly decreased in soil from columns with recycled materials compared to the control, reaching the minimum values in the MixC (11.6 g/kg and 13.8 g/kg, corresponding to -59% and -53% under standard and flooding conditions, respectively; Table S5). Total nitrogen also showed significant reductions of -34% and -38% in MixA, and -42% and -47% in MixC, under standard and flooding conditions, respectively (Table S5).

Compared to Lufa 2.2, Lufa 2.1 was characterized by lower values of organic carbon (12.3 g/kg) and total nitrogen (0.96 g/kg). After 28 days, a significant reduction of organic carbon compared to CT was observed in MixC flooding conditions (-49%), while an increase (even though not significant) was observed in MixA (+26% in standard conditions). Total nitrogen levels were significantly lower in MixC compared to CT, and in MixA under flooding conditions (Table S5).

Lufa 2.2 showed a higher content of total and organic phosphorus compared to Lufa 2.1, but lower available phosphorus (Table 4). After 28 days, available phosphorus in MixA and MixC was significantly lower than in CT, for both Lufa soils, with the strongest reduction observed in MixA -60% and -63%, under standard and flooding conditions, respectively. In Lufa 2.1, significant decreases in total and organic P was observed in MixA and MixC compared to CT, under flooding conditions. Similar but not significant reductions were also observed in standard conditions and in Lufa 2.2.

Table 4. Organic carbon (C), total nitrogen (N), available (Avail. P), organic (organic P) and total phosphorus (Total P) were measured in the beginning (day 0 -baseline status of the soils, measured on just one replicate) and in the end of experiment (day 28), (mean ± sd), grouped by Lufa type and moisture conditions.

Lufa	Condition	Treatment	Organic C (g/kg)	Total N (g/kg)	Avail. P (mg/kg)	Organic P (mg/kg)	Total P (mg/kg)
2.2	Baseline (day 0)	CT	28.7	2.55	15.4	493	645
	Standard	CT	28.7 ± 1.7	2.57 ± 0.14	16.6 ± 0.6	491 ± 46	644 ± 46
		MixA	21.1 ± 2.8	1.59 ± 0.10	6.6 ± 0.4	315 ± 179	443 ± 183
		MixC	11.6 ± 1.3	1.36 ± 0.03	11.7 ± 0.5	216 ± 27	398 ± 21
	Flooding	CT	29.4 ± 3.3	2.62 ± 0.27	13.4 ± 0.3	499 ± 40	636 ± 43
		MixA	24.0 ± 2.3	1.74 ± 0.12	4.9 ± 0.1	478 ± 319	604 ± 326
		MixC	13.8 ± 0.7	1.51 ± 0.07	11.4 ± 0.3	244 ± 16	439 ± 10
2.1	Baseline (day 0)	CT	12.3	0.96	71.3	229	535
	Standard	CT	10.3 ± 0.4	0.85 ± 0.02	58.0 ± 0.4	199 ± 17	500 ± 29
		MixA	13.1 ± 3.0	0.83 ± 0.07	27.7 ± 0.6	145 ± 50	446 ± 44
		MixC	7.0 ± 0.4	0.57 ± 0.03	41.5 ± 1.0	158 ± 31	501 ± 30
	Flooding	CT	11.6 ± 1.7	0.89 ± 0.11	56.2 ± 1.8	218 ± 21	547 ± 33
		MixA	12.9 ± 1.5	0.72 ± 0.01	25.1 ± 1.4	160 ± 7	464 ± 10
		MixC	5.9 ± 0.8	0.56 ± 0.02	44.0 ± 4.2	101 ± 29	463 ± 25

Assessment of Microbial Activities

Enzymatic activities

During the experiment, DHA activity increased in CT compared to day zero values (Figure 2). A similar, although less marked, increase was also observed in MixA, followed by a decrease on day 28 under flooding conditions. Overall, higher DHA values were observed in Lufa 2.2 and in standard moisture conditions for both CT and MixA. In contrast, MixC showed an immediate reduction in DHA activity, with values close to zero. Statistically significant differences were observed among all the treatments, with only two exceptions: CT *vs*. MixA after day 14 in standard moisture conditions, and MixA *vs*. MixC after day 28 under flooding conditions ($p<0.05$; Table S6).

Except for an initial increase in Lufa 2.2 control after 14 days under standard moisture conditions, ACP activity decreased in all the treatments compared to the initial values, with MixC showing the greatest reduction (Figure 2). Different trends were observed over time according to the soil moisture: ACP gradually decreased in standard moisture conditions, while it showed an increase in the last sampling time under flooding conditions. The differences among the treatments were statistically significant, except for CT vs. MixA with Lufa 2.2 on day 28 under flooding conditions, and between MixA and MixC with Lufa 2.1 on day 28 (Table S6).

ALP activity generally decreased in Lufa 2.2 treatments compared to day zero values, with MixC showing the strongest reduction (Figure 2). Over time, CT and MixC exhibited relatively stable values across sampling times, whereas MixA showed different trends according to soil moisture: ALP activity increased over time under standard conditions and decreased under flooding conditions. In Lufa 2.1, ALP activity increased significantly in MixA compared to initial values, exhibiting the same temporal patterns observed in Lufa 2.2, particularly in relation to moisture conditions. A less pronounced increase was also observed in MixC under flooding conditions. Statistically significant differences were observed among all the treatments, with few exceptions, especially in Lufa 2.1 ($p<005$; Table S6).

The trends in ARS and UR activity are not considered for Lufa 2.1 due to the high variability observed in the obtained values. In Lufa 2.2, ARS activity decreased compared to day zero in the treatments with recycled materials, with the strongest reduction in MixC (Figure 2). MixA exhibited the same temporal pattern observed in ALP activity, increasing over time in standard moisture and decreasing under flooding conditions. The same temporal trend was observed also for the control soil, whose highest values exceed the day zero levels. The differences between treatments were always statistically significant (Table S6). UR activity increased compared to day zero in CT and MixA, whereas it decreased in MixC with values close to zero, except in standard moisture after 28 days. The differences among the treatments were always statistically significant, except for CT vs. MixA on day 28 under standard moisture conditions.

Microbial basal respiration

Regarding Lufa 2.2 soil under standard conditions, slight differences in basal respiration were observed on day 14 samples after 216 hours, with higher microbial basal respiration in treatment MixA (600 mg/kg), followed by treatments CT (550 mg/kg) and MixC (500 mg/kg). On samples collected on day 28, a different trend was observed: microbial respiration from the MixC-treatment was initially lower than that observed in the CT and MixA-treatments (Figure 3), but it eventually reached the values of the CT (300 mg/kg), while the MixA treatment remained slightly lower (270 mg/kg). Under flooding conditions, soil from MixC treatments exhibited higher basal respiration across all time intervals on day 14 samples, reaching a cumulative value of 325 mg/kg after 216 hours, followed by MixA (215 mg/kg) and CT (180 mg/kg). On day 28, samples showed higher basal respiration (265 mg/kg after 216 h), followed by MixC and then MixA, which remained the lowest until 72 h (Figure 3); however, it reached values comparable to those of MixC (200 mg/kg).

On day 14, samples of Lufa 2.1 under standard conditions were higher in CT and MixA (245 mg/kg) than in MixC (185 mg/kg). On day 28, samples showed a similar trend, but with lower values: MixA and CT (210 and 190 mg/kg, respectively) are higher than MixC (115 mg/kg). Under flooding conditions, MixC showed the highest microbial respiration on day 14 (Figure 3), reaching a final cumulative value of 350 mg/kg, followed by MixA (180 mg/kg) and CT (110 mg/kg). On day 28, the basal respiration of microorganisms from the MixC treatment was lower across all time intervals (Figure 3), with a total cumulative value of 95 mg/kg. CT and MixA showed similar respiration values from the end of the experiment, with CT slightly higher (180 mg/kg) than MixA (150 mg/kg).

Generally, the statistical analysis of the respiration results at the last sampling interval (216 h) showed no significant differences between treatments, regardless of Lufa soil type, sampling time, or moisture regime (Table S7).

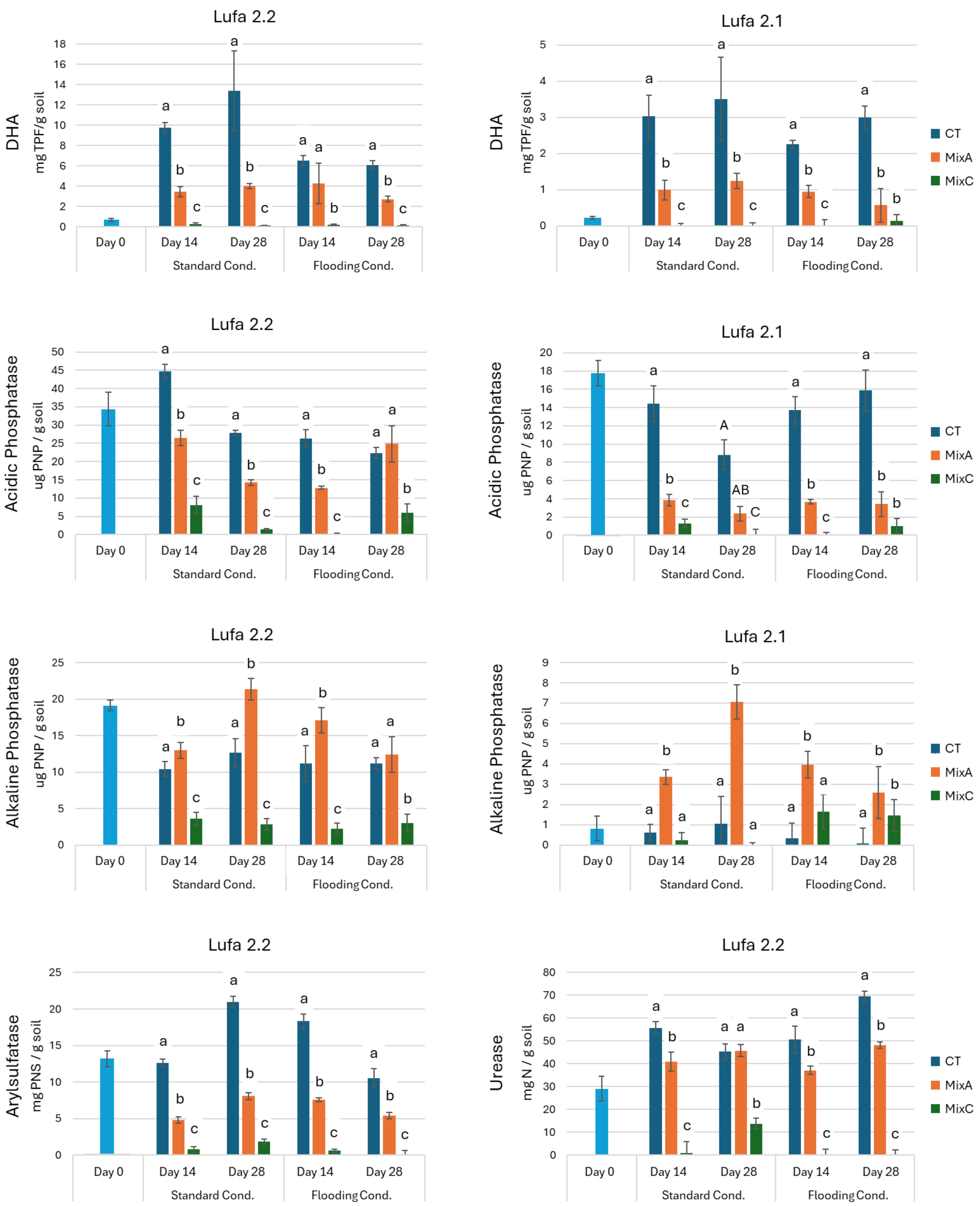


Figure 2. Enzymatic activities (DHA, ACP, ALP, ARS and UR) measured in Lufa soils (2.2 and 2.1), under moisture conditions (Standard and Flooding) and sampling time (day 0, 14 and 28). Different letters indicate significant differences ($p < 0.05$) among the treatments, within the same sampling time and moisture condition; uppercase letters refer to comparisons performed using Kruskal–Wallis analysis.

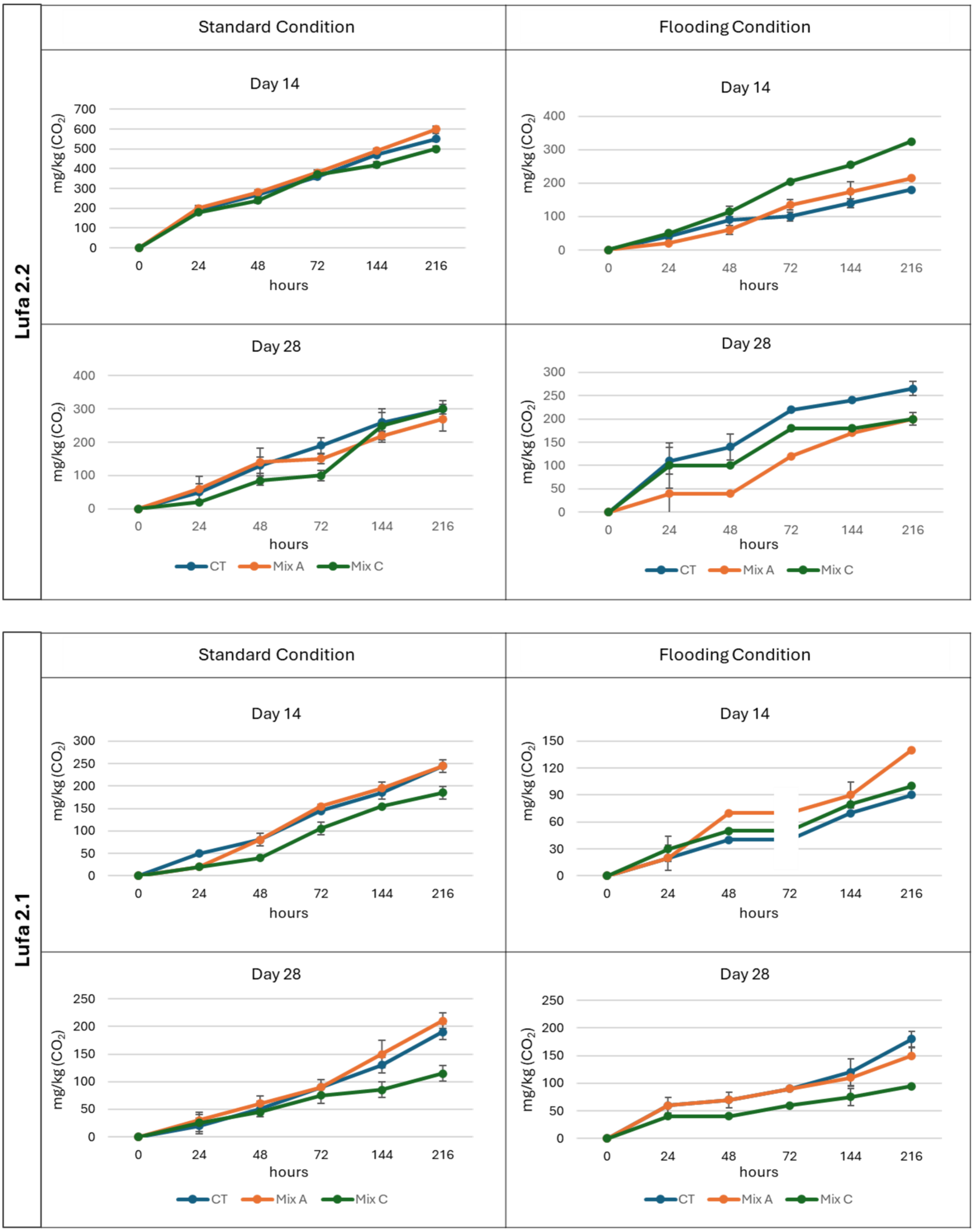


Figure 3. Respiration of Lufa 2.2 and Lufa 2.1. Missing values at reading 72 h from Lufa 2.1 treatments under flooding conditions, on day 14, were removed. No significant differences were detected between CT and treatments.

Phytotoxicity tests

The results of the phytotoxicity test, conducted using two types of Lufa soils and two plant species, are presented in Table 6. Results from statistical analysis are reported in Table S8 and S9.

In Lufa 2.2, shoot and root length of *M. sativa* and *L. perenne* generally showed reductions in the treatments with recycled materials compared to the control. Significant differences were observed between CT and MixC for *M. sativa* and *L. perenne* under standard moisture conditions. No differences were observed in *L. perenne* shoot length under flooding conditions. *M. sativa* root dry biomass significantly decreased in MixC under flooding conditions, while no significant differences were observed for shoot biomass. In contrast, *L.* perenne exhibited a significant increase in root biomass following the MixC treatment under flooding conditions, and a significant decrease in shoot biomass with standard moisture levels. No significant differences in biomass were observed between CT and MixA for any plant species tested.

In Lufa 2.1 soil, a slight increase in *M. sativa* shoot and root length was observed in MixA compared to CT (but not statistically significant). The same trend was observed in *L. perenne* root length, while shoot length didn't show a clear pattern. In contrast, an overall decrease in shoot and root length was observed in MixC for both plant species, with significant differences in root length under standard moisture conditions. Root dry biomass of *M. sativa* in MixC significantly decreased with standard moisture conditions, while it significantly increased under flooding conditions. *M. sativa* shoot biomass significantly increased in both MixA and MixC under flooding conditions. A significant increase was also observed in *L. perenne* shoot biomass in MixC under flooding conditions, whereas a significant decrease in both root and shoot biomass was detected in MixC with standard moisture.

The dry biomass root:shoot ratio (R:S) decreased across treatments with Lufa 2.2 soil (CT > MixA > MixC) for *M. sativa*, while it increased for *L. perenne* (MixC > MixA > CT) under both moisture conditions. In Lufa 2.1 experiments, R:S highest values were observed in MixA, while CT and MixC showed variable values according to the plant species and moisture conditions: R:S was higher in CT than in MixC in *M. sativa* standard moisture and *L. perenne* flooding conditions, while it was higher in MixC than in CT in *M. sativa* flooding conditions and *L. perenne* standard moisture conditions.

The Relative Seed Germination (RSG) in Lufa 2.2 experiments was generally high (93-100%) in CT and MixA, except for *L. perenne* under flooding conditions (67% CT; 80% MixA). In MixC, RSG was high for *L. perenne* (93-100%) and lower for *M. sativa* (80% with standard moisture, 60% in flooding conditions). In Lufa 2.1, the *M. sativa* RSG was higher in the standard treatment (93-100%) than in flooding conditions (80-87%) across all treatments. *L. perenne* RSG in CT and MixA was higher with standard moisture (87-93%) than under flooding condition (80%), while in MixC it remained constant (80%) regardless of soil moisture conditions.

In Lufa 2.2, the Relative Root Growth (RRG) was similar in MixA compared to the control, whereas a strong reduction was observed in MixC for both *M. sativa* (0-10%) and *L. perenne* (14-22%). In Lufa 2.1, root growth in MixA was higher than in the control, with RRG values of 126% in root biomass under standard moisture for both plant species, and even higher under flooding conditions (165% and 189% for *M. sativa* and *L. perenne*, respectively). In contrast, MixC exhibited a significant reduction in root growth, particularly in standard moisture levels (18-30%), except for *L. perenne* under flooding conditions.

The Germination Index (GI), which measures the overall impact of the recycled material on plant species development, showed negative effects in MixC (GI $\leq$ 0.6) across all plant species, Lufa soil types, and moisture conditions, except for *L. perenne* in Lufa 2.1 under flooding conditions (1.5). In Lufa 2.2, MixA showed a moderate increase in GI of *L. perenne* under flooding conditions, whereas in Lufa 2.1 an overall increase was observed for both plant species, especially under flooding conditions (GI $\geq$ 1.8).

Table 6. Measured parameters and calculated indices for the phytotoxicity test on *Medicago sativa* and *Lolium perenne*: shoot and root length (cm) and dry weight (mg); root:shoot ratio (R:S); seed germination (SG); relative seed germination (RSG); relative root growth (RRG); Germination Index (GI). Thresholds for RSG and RRG indicating relevant reductions or increases were set at ≤ 80% and ≥ 120%, respectively, GI values were interpreted as follows [25]: GI ≤ 0.5 (high negative impact); 0.5 < GI ≤ 0.8 (moderate negative impact); 0.8 < GI ≤ 1.2 (lack of relevant impact); 1.2 < GI ≤ 1.5 (moderate positive impact); GI > 1.5 (high positive impact).

Lufa	Plant	Moisture	Treatment	Length (cm)		Dry weight (mg)		Indices (%)				GI
				Shoot	Root	Shoot	Root	R:S	SG	RSG	RRG	
2.2	*M. sativa*	Standard	CT	4.3 ± 0.7	6.2 ± 2.3	6.6 ± 0.4	1.9 ± 0.2	29	100	100	100	1.0
			MixA	3.7 ± 1.1	6.0 ± 2.2	7.1 ± 0.4	2.0 ± 0.7	28	100	100	97	1.0
			MixC	2.1 ± 0.6	0.6 ± 0.5	6.5 ± 0.5	1.0 ± 0.6	15	80	↓ 80	↓ 10	0.1
		Flooding	CT	3.7 ± 0.6	4.9 ± 0.9	5.8 ± 1.0	2.0 ± 0.5	34	93	100	100	1.0
			MixA	3.3 ± 1.1	4.2 ± 2.1	5.3 ± 0.3	1.0 ± 0.3	19	100	107	86	0.9
			MixC	1.4 ± 0.5	0.0 ± 0.0	2.8 ± 1.6	0.0 ± 0.0	0	60	↓ 64	↓ 0	0.0
	L. perenne	Standard	CT	6.2 ± 1.4	5.1 ± 1.3	3.2 ± 0.3	6.3 ± 0.4	197	93	100	100	1.0
			MixA	5.2 ± 1.9	4.7 ± 1.6	2.3 ± 0.5	6.1 ± 0.4	265	100	107	92	1.0
			MixC	3.1 ± 1.9	1.1 ± 0.7	1.7 ± 0.3	6.8 ± 0.8	400	93	100	↓ 22	0.2
		Flooding	CT	3.7 ± 2.2	3.5 ± 1.9	1.3 ± 0.6	3.0 ± 1.1	231	67	100	100	1.0
			MixA	4.5 ± 1.8	4.0 ± 2.2	1.3 ± 0.3	4.2 ± 1.2	323	80	↑ 120	114	1.4
			MixC	4.3 ± 1.8	0.5 ± 0.4	1.9 ± 0.1	6.4 ± 0.4	337	100	↑ 150	↓ 14	0.2
2.1	*M. sativa*	Standard	CT	3.2 ± 0.7	5.7 ± 1.4	6.3 ± 0.4	2.1 ± 0.5	33	100	100	100	1.0
			MixA	3.7 ± 0.7	7.2 ± 1.6	6.3 ± 0.9	2.4 ± 0.2	38	100	100	↑ 126	1.3
			MixC	2.4 ± 0.5	1.0 ± 0.3	8.4 ± 3.5	0.5 ± 0.6	6	93	93	↓ 18	0.2
		Flooding	CT	1.8 ± 1.2	2.0 ± 1.0	2.9 ± 0.2	0.7 ± 0.5	24	80	100	100	1.0
			MixA	3.0 ± 1.6	3.3 ± 2.3	4.6 ± 0.8	1.8 ± 0.6	39	87	108	↑ 165	1.8
			MixC	3.7 ± 0.6	1.2 ± 0.3	5.6 ± 0.2	1.6 ± 0.3	29	80	100	↓ 60	0.6
	L. perenne	Standard	CT	5.8 ± 1.2	4.7 ± 1.0	2.6 ± 0.4	5.7 ± 1.0	219	87	100	100	1.0
			MixA	5.3 ± 0.9	5.9 ± 1.2	2.1 ± 0.2	6.4 ± 1.1	305	93	108	↑ 126	1.4
			MixC	4.5 ± 1.4	1.4 ± 0.8	1.7 ± 0.1	4.0 ± 1.0	235	80	92	↓ 30	0.3
		Flooding	CT	6.5 ± 2.9	2.8 ± 1.8	2.0 ± 0.9	4.3 ± 1.7	215	60	100	100	1.0
			MixA	5.8 ± 2.3	5.3 ± 2.3	2.2 ± 0.1	4.9 ± 0.6	223	73	↑ 122	↑ 189	2.3
			MixC	6.9 ± 0.8	3.1 ± 0.9	3.7 ± 0.5	5.6 ± 0.4	151	80	↑ 133	111	1.5

DISCUSSION

Effects on soil physicochemical properties

In the current study, a clear pattern emerged regarding the incorporation of RCA and RAP in standard soils, which immediately affected the soil's physicochemical properties, including water retention and compaction, pH, conductivity, and soil nutrient content.

In standard moisture conditions, both soils maintained stable water content and compaction, indicating a limited impact of the recycled material on pore structure and water-holding capacity. However, under flooding conditions, different behaviors were observed among treatments. Overall, soils mixed with recycled materials were less compacted compared to the control, MixA retained less water than CT, while MixC retained a similar or higher amount of water than CT. These results indicate that the incorporation of recycled materials in soil affects its granulometry and can potentially improve its physical behavior by modifying water-holding capacity and limiting compaction, as already demonstrated by other authors [5,10,11]. In particular, RAP coarse aggregates improve water drainage in soil, whereas RCA may enhance water retention, possibly due to finer particle content. Percolation patterns showed differences between Lufa soil types, highlighting the importance of initial soil properties, such as texture and organic matter content, on drainage behavior, regardless of recycled aggregate addition.

RCA and RAP additions resulted in a rapid and persistent pH increase in both soil and percolation water, particularly in MixC, indicating the release of alkaline compounds from the recycled materials. Similarly, the rapid increase in electrical conductivity indicates the release of soluble ions, especially from MixC [26]. However, the gradual decrease in EC of the percolation water over time suggests a progressive leaching of ions and stabilization of soil chemistry. These findings are in line with the analysis performed by the providers on RAP and RCA eluates (Appendix S1,Table AS1c and Appendix S2, Table AS2b, respectively), which showed a pH of 10.1 and 8.04 and an EC of 445 uS/cm and 113 uS/cm, respectively, and are easily explained by the chemical and mineralogical composition of the recycled materials. Although the composition of RCA largely depends on the source of the concrete, cement is the dominant component that governs its chemistry. Limestone, the base material for cement production, is composed of approximately 60% calcium carbonate, with the remaining 40% consisting of clay, silica, and dolomite [27]. As a result, RCA typically contains abundant minerals capable of releasing alkaline ions into the soil solution, which explains the observed increase in pH. Furthermore, RCA may contain soluble salts of potassium (K), iron (Fe), and cobalt (Co), contributing to an increase in electrical conductivity [27]. Similar considerations can be applied to RAP, since the aggregates used in asphalt production – the aggregates are combined with bitumen as a binder – have a mineralogical composition very similar to that of concrete (Appendix S1, Table AS1a).

The two soil types exhibited different nutrient content, which is critical for interpreting the effects of recycled material amendments on soil nutrient levels. Indeed, in Lufa 2.2, initially rich in organic carbon and total nitrogen, the addition of recycled materials resulted in an overall reduction of these components, whereas their levels in the control soil remained stable. In contrast, the low level of nutrient content in Lufa 2.1 showed increases in organic carbon and total nitrogen across all treatments, over time. Notably, the increase in soil organic carbon observed in MixA, is probably due to the presence of a RAP fine fraction (< 0.2 mm) that was collected and analyzed together with the soil sample. Regarding phosphorus content, the distribution among the different P forms indicated that Lufa 2.2 has a substantial reserve of total and organic P, but a relatively low amount is immediately available to plants. In contrast, Lufa 2.1 contains lower total and organic P, but a higher fraction in an available form. The addition of recycled materials resulted in a significant decrease in available phosphorus in both Lufa soils. This reduction can be attributed to the precipitation of phosphate ions, which react with calcium present in the aggregates to form calcium phosphates, especially under alkaline conditions [28].

Microbial communities' responses

Due to their close relationship with nutrient cycling, soil enzymatic activities are great indicators of soil quality and functionality [29]. The results of this experiment highlight the influence of RAP and RCA on microbial functioning, as well as the different moisture regimes. The increase in DHA activity in the control and, to a lesser extent, in MixA indicates stimulation of microbial activity following the changes in environmental conditions at the beginning of the experiment, as Lufa soils had been previously stored at 4°C. This effect was less pronounced under flooding conditions, likely due to reduced oxygen availability in saturated soils, which limits aerobic respiration and consequently oxidative enzymatic processes [30]. The almost complete inhibition of DHA in MixC highlights a strong inhibitory effect of RCA, likely due to an increase in soil pH beyond the optimal values, typically between 6.5 and 8 [15,31]. Soil pH is indeed one of the main factors shaping microbial community composition and functions [31,32].

Phosphatase activities (ACP and ALP) showed contrasting responses depending on soil moisture conditions and treatments. The general decrease in ACP might have resulted from the pH increase induced by the recycled materials, which both limits the enzymes' activities and reduces phosphorus availability [31]. In contrast, the ALP increase observed in MixA is probably due to the more favorable conditions (higher pH values compared to CT) for alkaline enzymes compared to the control soil [33]. The extremely low activity detected in MixC underscores the detrimental effect of RCA on both phosphatases.

The trends observed for the activities of both ARS and UR in Lufa 2.2 further support the interpretation provided for the previous enzymes. Indeed, both activities decreased in the treatments containing recycled materials, with MixC showing the lowest value.

Over time, the progressive increase generally observed in MixA and CT under standard moisture conditions, except for ACP, suggests an activity recovery and stabilization of the microbial community, supported by the favorable moisture conditions (increased soil pH).

The results of microbial basal respiration indicate that the addition of RCA and RAP had variable effects depending on soil type, treatment, and moisture regime. Despite transient variations, overall microbial respiration was not significantly affected, indicating that RCA and RAP at a 1:1 volumetric ratio with standard soils do not substantially alter basal microbial function.

Phytotoxic effects

The phytotoxicity tests highlight that the effects of RCA and RAP on early plant development are highly dependent on both the type of recycled material and the soil and moisture conditions.

Overall, MixC showed the strongest negative impacts on shoot and root growth, particularly under standard moisture, suggesting that RCA may release compounds or alter soil properties (e.g., significantly increasing pH) and inhibit plant development [15]. In contrast, MixA generally had neutral or even positive effects on both shoot and root growth, especially with Lufa 2.1 and under flooding conditions. This preference implies that the soil environment is relatively favorable for plants, allowing them to allocate more resources to aboveground biomass for photosynthesis and growth.

Changes in R:S ratios highlight the capacity of plants to dynamically adjust their growth strategies in response to the physical and chemical changes induced by recycled materials in soil. *M. sativa* generally favored root growth compared to shoots, especially in MixC. The preferential root growth suggests that *M. sativa* under MixC conditions needed to maximize water and nutrient uptake under less favorable conditions [34]. In contrast, preferential shoot growth was observed for *M. sativa* in MixA with Lufa 2.1 and for *L. perenne* in both MixA and MixC, regardless of soil type and moisture regime. This pattern implies that the soil environment was relatively favorable for plant development, allowing greater allocation of resources to aboveground biomass for photosynthesis and growth.

RSG and RRG indices indicate that both recycled materials primarily affect root growth compared to seed germination. Interestingly, *L. perenne* seeds germination appeared to be stimulated in both MixA and MixC under flooding conditions, possibly due to the better moisture and aeration conditions related to the presence of coarse aggregates, compared to the control. A strong negative impact of RCA on root growth was observed in all the MixC treatments, whereas RAP presence stimulated root growth in all the MixA treatments with Lufa 2.1.

Overall, the Germination Index (GI) confirms these patterns, showing strong inhibition of plant development in MixC and a moderate to strong stimulation in MixA under certain conditions, reinforcing the idea that recycled concrete may exert phytotoxic effects, whereas recycled asphalt is generally compatible with early plant growth [25].

The observed species-specific responses suggest differential tolerance and adaptation mechanisms between plant species [35].

CONCLUSION

This study confirmed that the incorporation of recycled materials such as RCA and RAP can significantly affect soil properties, microbial activity, and plant germination and growth. However, the direction and magnitude of these effects strongly depend on several factors, including the composition of the aggregates, the initial soil properties, and the prevailing environmental conditions.

Overall, recycled materials substantially modified soil physicochemical properties through changes in texture, alkalinity, and chemical composition. While such alterations may improve physical aspects, such as drainage and compaction, they can also impact soil chemistry (*e.g.*, a significant increase in pH and EC), sometimes negatively affecting microbial activity and plant responses. RCA and RAP strongly influenced soil nutrient content and dynamics, with effects primarily depending on initial soil fertility and the composition of recycled materials. Nutrient-rich soils are negatively affected, whereas nutrient-poor soils may benefit from the addition of carbon and nitrogen. Phosphorus availability appears particularly sensitive to these treatments.

Soil enzymatic activities were significantly altered by the addition of recycled materials, with the strongest detrimental effects observed in the presence of RCA. Enzymes involved in carbon and sulfur cycling appeared particularly sensitive, while those related to phosphorus were more resilient, especially with RAP, which might be due to the alkaline conditions in soil.

The non-target effect of materials tested in the phytotoxic test showed that the incorporation of RCA in soil may pose risks to early plant development due to its alkalinity and soluble compounds, while RAP appears to be more suitable for reuse in soil, promoting plant growth under certain conditions.

These results emphasize that the suitability of recycled materials for soil applications must be carefully evaluated. Further studies are necessary to test other experimental conditions and factors (*e.g.* RCA and RAP concentrations and granulometry, temperature and moisture conditions, and different vegetation cover) to optimize the use of recycled materials in soil, avoiding their negative effects.

Bibliography

1. European Commission; Directorate-General for Internal Market, I.E. and Sme.; Oberender, A.; Fruergaard Astrup, T.; Frydkjær Witte, S.; Camboni, M.; Chiabrando, F.; Hayleck, M.; Akelytė, R. *EU Construction & Demolition Waste Management Protocol Including Guidelines for Pre-Demolition and Pre-Renovation Audits of Construction Works – Updated Edition 2024*; Publications Office of the European Union, 2024;
2. Eurostat Waste Statistics - Statistics Explained Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Waste_statistics (accessed on 29 October 2025).
3. Damgaard, A.; Lodato, C.; Butera, S.; Fruergaard, T.F.; Kamps, M.; Corbin, L.; Tonini, D.; Astrup, T.F. Background Data Collection and Life Cycle Assessment for Construction and Demolition Waste (CDW) Management. *JRC130992* 2022, doi:10.2760/772724.
4. Rompato, B.; Rossi, R.; Giagnoni, L.; Mastrolonardo, G.; Argenti, G.; Staglianò, N.; Nuñez, R.P.; Certini, G. Removed Asphalt Can Be Used as a Component of Constructed Soils for Urban Greenery. *J Soils Sediments* 2025, doi:10.1007/s11368-024-03952-1.
5. Mikajlo, I.; Pando, A.; Robain, H.; Lerch, T.Z. Reusing Asphalt Millings with Excavated Materials and Compost to Construct Technosols: Effects on Soil Properties and Plant Growth. *J Soils Sediments* 2024, doi:10.1007/s11368-024-03942-3.
6. Reclaimed Asphalt Pavement - Material Description - User Guidelines for Waste and Byproduct Materials in Pavement Construction - FHWA-RD-97-148 Available online: https://www.fhwa.dot.gov/publications/research/infrastructure/pavements/97148/046.cfm (accessed on 29 October 2025).
7. Zhao, Z.; Xiao, F.; Amirkhanian, S. Recent Applications of Waste Solid Materials in Pavement Engineering. *Waste Management* 2020, *108*, 78–105, doi:10.1016/j.wasman.2020.04.024.
8. Tsakoumaki, M.; Plati, C. A Critical Overview of Using Reclaimed Asphalt Pavement (RAP) in Road Pavement Construction. *Infrastructures (Basel)* 2024, *9*, 128, doi:10.3390/infrastructures9080128.
9. Coull, M.; Butler, B.; Hough, R.; Beesley, L. A Geochemical and Agronomic Evaluation of Technosols Made from Construction and Demolition Fines Mixed with Green Waste Compost. *Agronomy* 2021, *11*, doi:10.3390/agronomy11040649.
10. Mastrolando, G.; Giagnoni, L.; Maienza, A.; Fantoni, G.; Rompato, B.; Argenti, G.; Selvi, F.; Fiore, B.; Certini, G. Use of Waste Materials as Feasible Strategy for Urban De-Sealed Soil Reclamation:

Evidence from a Restoration Case Study in Prato, Italy. In Proceedings of the 13th International Symposium of the International Union of Soil Sciences on Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA 13); 2025; pp. 158–159.
11. Yilmaz, D.; Cannavo, P.; Séré, G.; Vidal-Beaudet, L.; Legret, M.; Damas, O.; Peyneau, P.-E. Physical Properties of Structural Soils Containing Waste Materials to Achieve Urban Greening. *J Soils Sediments* 2018, *18*, 442–455, doi:10.1007/s11368-016-1524-0.
12. Herbreteaua, A.; Schwartzb, C.; Beguiristaina, T.; Bojicc, C.; Cossu-Leguillec, C.; Durandb, A.; Leglizeb, P.; Lorgeouxd, C.; Faurea, P. Reactivity of Road Asphalt Buried in Soils after De-Sealing Operations. In Proceedings of the 13th International Symposium of the International Union of Soil Sciences on Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA 13); 2025; pp. 205–206.
13. Vytlacilova, V. Testing Ecological Suitability for the Utilization of Recycled Aggregates. *Green Processing and Synthesis* 2017, *6*, 225–234, doi:10.1515/gps-2016-0074.
14. Rodrigues, P.; Silvestre, J.D.; Flores-Colen, I.; Viegas, C.A.; Ahmed, H.H.; Kurda, R.; de Brito, J. Evaluation of the Ecotoxicological Potential of Fly Ash and Recycled Concrete Aggregates Use in Concrete. *Applied Sciences* 2020, *10*, 351, doi:10.3390/app10010351.
15. Mariaková, D.; Mocová, K.A.; Pešta, J.; Fořtová, K.; Tripathi, B.; Pavlů, T.; Hájek, P. Ecotoxicity of Concrete Containing Fine-Recycled Aggregate: Effect on Photosynthetic Pigments, Soil Enzymatic Activity and Carbonation Process. *Sustainability* 2022, *14*, 1732, doi:10.3390/su14031732.
16. Geitner, N.K.; Ogilvie Hendren, C.; Cornelis, G.; Kaegi, R.; Lead, J.R.; Lowry, G. V.; Lynch, I.; Nowack, B.; Petersen, E.; Bernhardt, E.; et al. Harmonizing across Environmental Nanomaterial Testing Media for Increased Comparability of Nanomaterial Datasets. *Environ Sci Nano* 2020, *7*, 13–36, doi:10.1039/C9EN00448C.
17. International Organization for Standardization *ISO 10390:2021 – Soil, Treated Biowaste and Sludge – Determination of PH*; Geneva, Switzerland, 2021;
18. D.M. n. 186 - Min. Dell'Ambiente e Della Tutela Del Territorio Decreto 05/04/06 - Tab. p. m; Regolamento UE N. 1357/2014 Della Commissione Del 18 Dicembre 2014.
19. Dick, R.P.; Breakwell, D.P.; Turco, R.F. Soil Enzyme Activities and Biodiversity Measurements as Integrative Microbiological Indicators. In; 2015; pp. 247–271.
20. Loureiro, S.; Nogueira, A.J.A.; Soares, A.M.V.M. A Microbial Approach in Soils from Contaminated Mine Areas: The Jales Mine (Portugal) Case Study. *Fresenius Environ Bull* 2007.
21. FAO *Standard Operating Procedure for Soil Respiration Rate*; Rome, 2023;
22. International Organization for Standardization *ISO 18763:2016 – Soil Quality: Determination of the Toxic Effects of Pollutants on Germination and Early Growth of Higher Plants*; Geneva, Switzerland, 2016;
23. Malheiro, C.; Prodana, M.; Patinha, C.; Morgado, R.G.; Loureiro, S. Insect Frass from Upcycling Vegetable By-Products with Cereals: Effects on the Soil Properties, Plant Development and Soil Invertebrate Fitness. *J Environ Manage* 2024, *372*, 123234, doi:10.1016/j.jenvman.2024.123234.
24. Luo, Y.; Liang, J.; Zeng, G.; Chen, M.; Mo, D.; Li, G.; Zhang, D. Seed Germination Test for Toxicity Evaluation of Compost: Its Roles, Problems and Prospects. *Waste Management* 2018, *71*, 109–114, doi:10.1016/j.wasman.2017.09.023.
25. Ranucci, E.; Treccani, S.; Ferruti, P.; Alongi, J. The Seed Germination Test as a Valuable Tool for the Short-Term Phytotoxicity Screening of Water-Soluble Polyamidoamines. *Polymers (Basel)* 2024, *16*, 1744, doi:10.3390/polym16121744.
26. Ekström, T. Leaching of Concrete : The Leaching Process and Its Effects. Doctoral Thesis (monograph), Lund University, 2003.
27. Sánchez-Cotte, E.H.; Pacheco-Bustos, C.A.; Fonseca, A.; Triana, Y.P.; Mercado, R.; Yepes-Martínez, J.; Lagares Espinoza, R.G. The Chemical-Mineralogical Characterization of Recycled Concrete Aggregates from Different Sources and Their Potential Reactions in Asphalt Mixtures. *Materials* 2020, *13*, 5592, doi:10.3390/ma13245592.
28. Avramenko, M. Soil Improvement Using Calcium Phosphate Compounds as a Novel Sustainable Method: A Review. *International Journal of GEOMATE* 2023, *24*, doi:10.21660/2023.101.g12142.
29. Peixoto, S.; Khodaparast, Z.; Cornelis, G.; Lahive, E.; Green Etxabe, A.; Baccaro, M.; Papadiamantis, A.G.; Gonçalves, S.F.; Lynch, I.; Busquets-Fite, M.; et al. Impact of Ag2S NPs on Soil Bacterial Community – A Terrestrial Mesocosm Approach. *Ecotoxicol Environ Saf* 2020, *206*, 111405, doi:10.1016/j.ecoenv.2020.111405.

30. Das, A.K.; Lee, D.-S.; Woo, Y.-J.; Sultana, S.; Mahmud, A.; Yun, B.-W. The Impact of Flooding on Soil Microbial Communities and Their Functions: A Review. *Stresses* 2025, *5*, 30, doi:10.3390/stresses5020030.
31. Qu, Y.; Tang, J.; Li, Z.; Zhou, Z.; Wang, J.; Wang, S.; Cao, Y. Soil Enzyme Activity and Microbial Metabolic Function Diversity in Soda Saline–Alkali Rice Paddy Fields of Northeast China. *Sustainability* 2020, *12*, 10095, doi:10.3390/su122310095.
32. Qi, D.; Wieneke, X.; Tao, J.; Zhou, X.; Desilva, U. Soil PH Is the Primary Factor Correlating With Soil Microbiome in Karst Rocky Desertification Regions in the Wushan County, Chongqing, China. *Front Microbiol* 2018, *9*, doi:10.3389/fmicb.2018.01027.
33. Campdelacreu Rocabruna, P.; Domene, X.; Preece, C.; Peñuelas, J. Relationship among Soil Biophysicochemical Properties, Agricultural Practices and Climate Factors Influencing Soil Phosphatase Activity in Agricultural Land. *Agriculture* 2024, *14*, 288, doi:10.3390/agriculture14020288.
34. Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass Allocation to Leaves, Stems and Roots: Meta-analyses of Interspecific Variation and Environmental Control. *New Phytologist* 2012, *193*, 30–50, doi:10.1111/j.1469-8137.2011.03952.x.
35. Chaffai, R.; Ganesan, M.; Cherif, A. Plant Response and Tolerance to Environmental Stresses. In *Plant Adaptation to Abiotic Stress: From Signaling Pathways and Microbiomes to Molecular Mechanisms*; Springer Nature Singapore: Singapore, 2024; pp. 31–47.

Supplementary materials

List of Figures

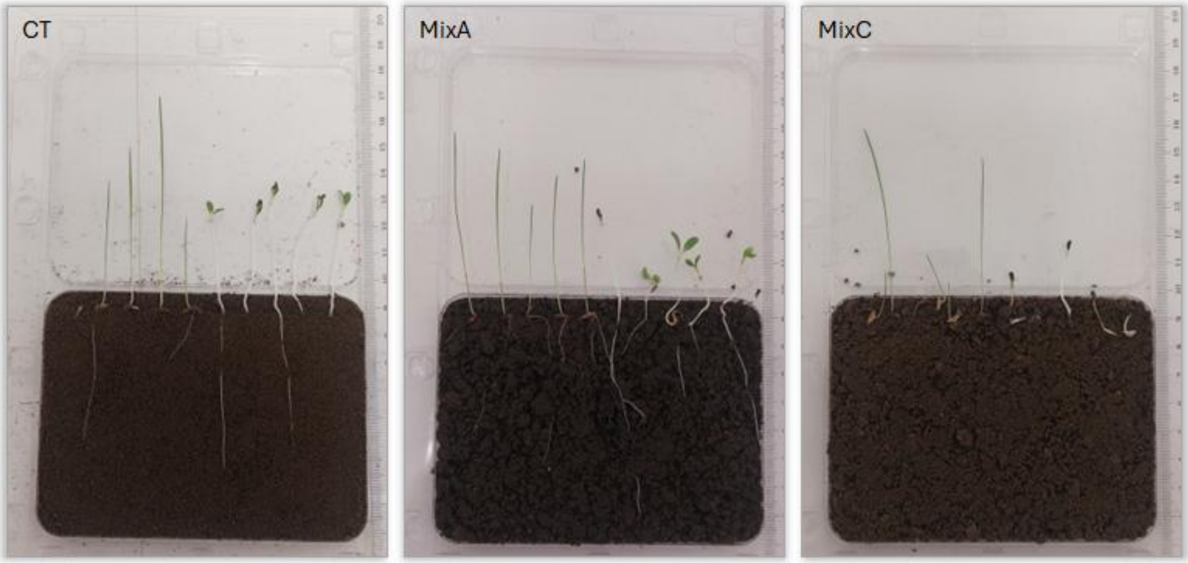


Figure S1. Example of plates used for the phytotoxic test, for each treatment (CT, MixA, MixC) with Lufa 2.2 in standard moisture conditions.

List of Tables

Appendix S1. Characteristics of Reclaimed Asphalt Pavement (RAP) provided by Edilnapoli Srl.

Table AS1a. *Aggregates composition characterization.*

Category	Values (%)
Calcareous sedimentary rocks	51%
Intrusive rocks (granitoid and gneiss)	16%
Effusive rocks (porphyry and basalt)	12%
Quartz, quartzites and flint	10%
Arenaceous sedimentary rocks (sandstone)	8%
Metamorphic rocks (gabbro)	3%

Table AS1b. *Chemical characterization of aggregates.*

Parameter	Values	Method	Limit values (DM 69, 2018)
Benzo(a)anthracene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Benzo(a)pyrene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Benzo(b)fluoranthene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Benzo(k)fluoranthene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Benzo(g,h,i)perylene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Chrysene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Dibenzo(a,l)pyrene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Dibenzo(a,e)pyrene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-

Dibenzo(a,i)pyrene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Dibenzo(a,h)pyrene	< 0,5 mg/kg	EPA 3541; EPA 8270D	-
Total PAH	< 5 mg/kg	EPA 3541; EPA 8270D	100 mg/kg
Asbestos	< 100 mg/kg	DM 06/09/94 Ali. 1 Met. B GU n.288 10/12/94	1000 mg/kg

Table AS1c. *Leaching characterization.*

Parameter	Method	Values	Limit values (DM 69, 2018)
pH	APAT CNR-IRSA Metodi analitici per le acque Vol.1 Met. 2060 (2003)	8.04	-
EC	APAT CNR-IRSA Metodi analitici per le acque Vol.1 Met. 2030 (2003)	113 uS/cm	-
Nitrates	APAT CNR-IRSA Metodi analitici per le acque Vol.2 Met. 4020 (2003)	1.29 mg/l	50 mg/l
Fluorides	APAT CNR-IRSA Metodi analitici per le acque Vol.2 Met. 4020 (2003)	0.21 mg/l	1.5 mg/l
Sulfate	APAT CNR-IRSA Metodi analitici per le acque Vol.2 Met. 4020 (2003)	23.9 mg/l	250 mg/l
Chlorides	APAT CNR-IRSA Metodi analitici per le acque Vol.2 Met. 4020 (2003)	4.74 mg/l	100 mg/l
Barium	UNI EN ISO 11885:2009	< 0.01 mg/l	1 mg/l
Copper	UNI EN ISO 11885:2009	< 0.01 mg/l	0.05 mg/l
Zinc	UNI EN ISO 11885:2009	< 0.01 mg/l	3 mg/l
Cobalt	UNI EN ISO 11885:2009	< 5 ug/l	250 ug/l
Nickel	UNI EN ISO 11885:2009	< 5 ug/l	10 ug/l
Arsenic	UNI EN ISO 11885:2009	<10 ug/l	50 ug/l
Cadmium	UNI EN ISO 11885:2009	< 1 ug/l	5 ug/l
Chromium (tot)	UNI EN ISO 11885:2009	<10 ug/l	50 ug/l
Lead	UNI EN ISO 11885:2009	< 10 ug/l	50 ug/l
Selenium	UNI EN ISO 11885:2009	< 5 ug/l	10 ug/l
Mercury	UNI EN ISO 11885:2009	< 1 ug/l	1 ug/l
Vanadium	UNI EN ISO 11885:2009	16 ug/l	250 ug/l
Beryllium	UNI EN ISO 11885:2009	< 20 ug/l	10 ug/l
Cyanides	APAT CNR-IRSA Metodi analitici per le acque Vol.2 Met. 4070 (2003)	< 20 ug/l	50 ug/l
COD	APAT CNR-IRSA Metodi analitici per le acque Vol.2 Met. 45130 (2003)	< 15 mgO_2/l	30 mgO_2/l

Appendix S2. Characteristics of Recycled Concrete Aggregates (RCA) provided by Sereca Sas.

Table AS2a. *Chemical characterization* of Recycled Concrete Aggregates.

Parameter	Method	Values (%)
Sulfates (soluble)	UNI EN 1744-1 .11	0.060 %
Sulfur (total)	UNI EN 13285	0.083 %

Table AS2b. *Characterization of RCA in Leaching.*

Parameter	Method	Unit	Values	Limit values
pH	UNI EN ISO 10523:2012	-	10.1	5.5-12.0
EC	UNI EN 27888:1995	mS/cm	0.445	-
Nitrates	UNI EN ISO 10304- 1 :2009	mg/I	1.66	50
Fluorides	UNI EN ISO 10304- 1 :2009	mg/I	< 0.1	1.5
Sulfate	UNI EN ISO 10304-1 :2009	mg/I	59.3	250
Chlorides	UNI EN ISO 10304-1 :2009	mg/I	4.61	100
Barium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	mg/I	0,000	1
Copper	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	mg/I	0.007	0.05
Zinc	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	mg/I	< 0.001	3
Cobalt	UNI EN ISO 15587- 1:2002 + UNI EN ISO 17294-2:2016	µg/I	< 10	250
Nickel	UNI EN ISO 15587- 1:2002 + UNI EN ISO 17294-2:2016	µg/I	< 1	10
Arsenic	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/I	< 10	50
Cadmium	UNI EN ISO 15587- 1:2002 + UNI EN ISO 17294-2:2016	µg/I	< 1	5
Chromium (tot)	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/I	20.3	50
Lead	UNI EN ISO 15587- 1:2002 +UNI EN	µg/I	< 10	50
Selenium	UNI EN ISO 15587- 1:2002 + UNI EN ISO 17294-2:2016	µg/I	< 2	10
Mercury	UNI EN ISO 15587-1:2002 +UNI EN	µg/I	< 0.5	1
Vanadium	UNI EN ISO 15587- 1:2002 +UNI EN	µg/I	12.0	250
Beryllium	UNI EN ISO 15587-1:2002 + UNI EN ISO 17294-2:2016	µg/I	< 1	10
Cyanides	APAT IRSA CNR 4070	µg/I	< 10	50
COD	DM 0610911994 MOCF	mg/I	< 5	30
pH	ISO 15705:2002	mgO2/l	< 5	30

Table S1. Statistical significance of soil moisture and compaction for each treatment (CT, MixA, or MixC) after 14 and 28 days of exposure. A one-way ANOVA followed by Tukey's post hoc test was used for multiple comparisons. Asterisk (*) indicates the statistical method used (Kruskal-Wallis One-Way Analysis of Variance on Ranks) when the ANOVA assumptions fail.

Parameter	Lufa	Day	Moisture	F	P	Comparison	P
Moisture	**2.2**	day 14	Standard	4.650	0.065	-	-
			Flooding	8.020	0.020	CT vs. MixA	0.019
						CT vs. MixC	0.572
						MixC vs. MixA	0.068
		day 28	Standard	6.661	0.030	MixC vs. MixA	0.025
						MixC vs. CT	0.226
						CT vs. MixA	0.255
			Flooding *	-	-	CT vs MixA	-
						CT vs MixC	-
						MixC vs MixA	-
	2.1	day 14	Standard	13.661	0.006	MixC vs. CT	0.007
						MixC vs. MixA	0.014
						MixA vs. CT	0.790
			Flooding	12.304	0.008	MixC vs. MixA	0.008
						MixC vs. CT	0.025
						CT vs. MixA	0.546
		day 28	Standard	27.618	<0.001	MixC vs. MixA	0.002
						MixC vs. CT	0.002
						CT vs. MixA	0.974
			Flooding	3.644	0.092	-	-
Compaction	**2.2**	day 28	Standard	27.650	<0.001	MixC vs. MixA	<0.001
						MixC vs. CT	0.054
						CT vs. MixA	0.011
			Flooding	16.117	0.004	MixA vs. CT	0.004
						MixA vs. MixC	0.401
						MixC vs. CT	0.016
	2.1	day 28	Standard	0.430	0.673	-	-
			Flooding	191.625	<0.001	MixA vs. CT	<0.001
						MixA vs. MixC	0.003
						MixC vs. CT	<0.001

Table S2. The statistical significance of percolation water parameters was assessed for each soil treatment (CT, MixA, or MixC), including the collected volume (mL), pH, and electrical conductivity (EC).

Parameter	Lufa	Day	F	P	Comparison	P
Collected volume - mL	**2.2**	day 1	2.916	0.130	-	-
		day14	0.244	0.791	-	-
		day 15	0.974	0.430	-	-
		day 28	7.561	0.023	CT vs. MixA	0.024
					CT vs. MixC	0.063
					MixC vs. MixA	0.711
	2.1	day 1	0.845	0.475	-	-
		day14	0.064	0.938	-	-

		day 15	0.780	0.926	-	-
		day 28	1.285	0.343	-	-
pH	**2.2**	day 1	326.071	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	0.002
					MixA vs. CT	<0.001
		day14	24.566	0.001	MixA vs. CT	0.001
					MixA vs. MixC	0.037
					MixC vs. CT	0.024
		day 15	15.914	0.004	MixC vs. CT	0.005
					MixC vs. MixA	0.010
					MixA vs. CT	0.759
		day 28	29.809	<0.001	MixA vs. CT	<0.001
					MixA vs. MixC	0.076
					MixC vs. CT	0.007
	2.1	day 1	143.739	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	<0.001
					MixA vs. CT	<0.001
		day14	474.114	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	0.411
					MixA vs. CT	<0.001
		day 15	77.822	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	0.780
					MixA vs. CT	<0.001
		day 28	209.918	<0.001	MixA vs. CT	<0.001
					MixA vs. MixC	0.720
					MixC vs. CT	<0.001
EC	**2.2**	day 1	12.124	0.008	MixA vs. CT	0.006
					MixA vs. MixC	0.112
					MixC vs. CT	0.103
		day14	122.730	<0.001	MixA vs. CT	<0.001
					MixA vs. MixC	0.871
					MixC vs. CT	<0.001
		day 15	49.527	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	0.007
					MixA vs. CT	0.006
		day 28	117.169	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	0.003
					MixA vs. CT	<0.001
	2.1	day 1	46.672	<0.001	MixA vs. CT	<0.001
					MixA vs. MixC	0.107
					MixC vs. CT	0.001
		day14	278.852	<0.001	MixA vs. CT	<0.001
					MixA vs. MixC	0.122
					MixC vs. CT	<0.001
		day 15	373.957	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	<0.001
					MixA vs. CT	<0.001
		day 28	201.362	<0.001	MixC vs. CT	<0.001
					MixC vs. MixA	0.003
					MixA vs. CT	<0.001

Table S3. Soil pH and electrical conductivity (EC) values (mean ± sd) measured for each soil treatment (CT, MixA, MixC) at the initial time (day zero), along with the corresponding statistical significance.

Parameter	Lufa	Treatment	mean ± sd	F	P	Comparison	P
pH	**2.2**	CT	6.3 ± 0.04	902.0	<0.001	MixC vs. CT	<0.001
		MixA	7.6 ± 0.11			MixC vs. MixA	<0.001
		MixC	10.4 ± 0.17			MixA vs. CT	<0.001
	2.1	Ct	5.4 ± 0.09	667.1	<0.001	MixC vs. CT	<0.001
		MixA	7.6 ± 0.03			MixC vs. MixA	<0.001
		MixC	10.7 ± 0.29			MixA vs. CT	<0.001
EC	**2.2**	Ct	58 ± 0.9	66.0	<0.001	MixC vs. CT	<0.001
		MixA	182 ± 14.2			MixC vs. MixA	0.199
		MixC	210 ± 26.3			MixA vs. CT	<0.001
	2.1	CT	39 ± 6.0	153.2	<0.001	MixC vs. CT	<0.001
		MixA	154 ± 13.0			MixC vs. MixA	<0.001
		MixC	293 ± 27.3			MixA vs. CT	<0.001

Table S4. Statistical significance of soil pH and electrical conductivity (EC) measured for each soil treatment (CT, MixA or MixC) at days 14 and 28.

Parameter	Lufa	Day	Moisture	F	P	Comparison	P
pH	**2.2**	day 14	Standard	4.2	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
			Flooding	1,705.6	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
		day 28	Standard	19,108.4	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
			Flooding	2,386.2	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
	2.1	day 14	Standard	16.1	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
			Flooding	9.7	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
		day 28	Standard	6,596.8	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
			Flooding	3,397.4	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
EC	**2.2**	day 14	Standard	395.8	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
			Flooding	327.4	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001

						MixA vs. CT	<0.001
		day 28	Standard	54.9	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	0.002
						MixA vs. CT	0.013
			Flooding	3,006.5	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
	2.1	day 14	Standard	1,111.7	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
			Flooding	471.5	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	0.002
		day 28	Standard	601.1	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	<0.001
			Flooding	194.0	<0.001	MixC vs. CT	<0.001
						MixC vs. MixA	<0.001
						MixA vs. CT	0.004

Table S5. Statistical significance of soil nutrient content measured for each soil treatment (CT, MixA or MixC) at day 28.

Soil nutrient	**Lufa**	**Moisture**	**F**	**P**	**Comparison**	**P**
Organic C	**2.2**	Standard	51.0	<0.001	CT vs. MixC	<0.001
					CT vs. MixA	0.010
					MixA vs. MixC	0.004
		Flooding	22.0	0.002	CT vs. MixC	0.002
					CT vs. MixA	0.141
					MixA vs. MixC	0.012
	2.1	Standard	6.4	0.032	MixA vs. MixC	0.027
					MixA vs. CT	0.314
					CT vs. MixC	0.199
		Flooding	14.3	0.005	MixA vs. MixC	0.006
					MixA vs. CT	0.651
					CT vs. MixC	0.015
Tot al N	**2.2**	Standard	118.9	<0.001	CT vs. MixC	<0.001
					CT vs. MixA	<0.001
					MixA vs. MixC	0.072
		Flooding	33.2	<0.001	CT vs. MixC	<0.001
					CT vs. MixA	0.002
					MixA vs. MixC	0.304
	2.1	Standard	34.0	<0.001	CT vs. MixC	<0.001
					CT vs. MixA	0.789
					MixA vs. MixC	0.001
		Flooding	18.1	0.003	CT vs. MixC	0.002
					CT vs. MixA	0.048
					MixA vs. MixC	0.061
Available P	**2.2**	Standard	281.0	<0.001	CT vs. MixA	<0.001
					CT vs. MixC	<0.001
					MixC vs. MixA	<0.001

		Flooding	750.2	<0.001	CT vs. MixA	<0.001
					CT vs. MixC	<0.001
					MixC vs. MixA	<0.001
	2.1	Standard	1,248.7	<0.001	CT vs. MixA	<0.001
					CT vs. MixC	<0.001
					MixC vs. MixA	<0.001
		Flooding	98.6	<0.001	CT vs. MixA	<0.001
					CT vs. MixC	0.004
					MixC vs. MixA	<0.001
Organic P	**2.2**	Standard	5.0	0.053	-	-
		Flooding	3.2	0.111	-	-
	2.1	Standard	2.0	0.218	-	-
		Flooding	23.5	0.001	CT vs. MixC	0.001
					CT vs. MixA	0.033
					MixA vs. MixC	0.033
Total P	**2.2**	Standard	4.3	0.069	-	-
		Flooding	4.1	0.076	-	-
	2.1	Standard	2.6	0.153	-	-
		Flooding	11.5	0.009	CT vs. MixC	0.014
					CT vs. MixA	0.015
					MixA vs. MixC	0.999

Table S6. Statistical significance of soil enzymatic activities measured in each treatment (CT, MixA or MixC) after days 14 and 28 (*Kruskal-Wallis One Way Analysis of Variance on Ranks).

Enzyme	Lufa	Day	Moisture	F	P	Comparison	P
DHA	2.2	day 14	Standard	565.9	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
			Flooding	15.6	<0.001	CT vs. MixC	0.004
						CT vs. MixA	0.174
						MixA vs. MixC	0.032
		day 28	Standard	49.4		CT vs. MixC	<0.001
						CT vs. MixA	0.007
						MixA vs. MixC	0.006
			Flooding	251.7	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
	2.1	day 14	Standard	105.7	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	0.002
						MixA vs. MixC	<0.001
			Flooding	320.3	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
		day 28	Standard	56.2	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	0.012
						MixA vs. MixC	0.002
			Flooding	56.6	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	0.331
ACP	2.2	day 14	Standard	452.1	<0.001	CT vs. MixC	<0.001

						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
			Flooding	747.9	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
		day 28	Standard	2,242.8	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
			Flooding	79.0	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	0.353
						CT vs. MixC	<0.001
	2.1	day 14	Standard	156.7	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	0.004
			Flooding	1,007.7	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
		day 28	Standard *	-	-	CT vs MixC	
						CT vs MixA	
						MixA vs MixC	
			Flooding	138.9	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	0.100
ALP	2.2	day 14	Standard	102.4	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	0.021
						CT vs. MixC	<0.001
			Flooding	181.9	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	<0.001
						CT vs. MixC	<0.001
		day 28	Standard	266.5	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	<0.001
						CT vs. MixC	<0.001
			Flooding	79.0	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	0.353
						CT vs. MixC	<0.001
	2.1	day 14	Standard	104.3	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	<0.001
						CT vs. MixC	0.322
			Flooding	26.9	<0.001	MixA vs. CT	0.001
						MixA vs. MixC	0.008
						MixC vs. CT	0.106
		day 28	Standard	70.3	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	<0.001
						CT vs. MixC	0.383
			Flooding	19.7	0.002	MixA vs. CT	0.002
						MixA vs. MixC	0.057
						MixC vs. CT	0.038
ARS	2.2	day 14	Standard	844.9	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
			Flooding	1,246.1	<0.001	CT vs. MixC	<0.001

						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
		day 28	Standard	965.1	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001
			Flooding	102.1	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	0.001
						MixA vs. MixC	0.001
UR	2.2	day 14	Standard	134.6	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	0.010
						MixA vs. MixC	<0.001
			Flooding	214.2	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	0.004
						MixA vs. MixC	<0.001
		day 28	Standard	124.0	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	0.982
						CT vs. MixC	<0.001
			Flooding	1,002.4	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	<0.001
						MixA vs. MixC	<0.001

Table S7. Statistical significance in microbial basal respiration for each soil treatment (CT, MixA, MixC) at final reading 216 h, from soil sampled after 14 and 28 days of exposure (*Kruskal–Wallis one-way analysis of variance on ranks).

Lufa	Day	Moisture	F	P
2.2	day14	Standard	1.32	0.336
		Flooding *	-	-
	day28	Standard	0.09	0.914
		Flooding	0.23	0.806
2.1	day14	Standard	2.33	0.178
		Flooding *	-	-
	day28	Standard	4.50	0.064
		Flooding	5.03	0.880

Table S8. Statistical significance for plant-related parameters for *Medicago sativa* (*i.e.*, root and shoot length and dry weight) measured in the phytotoxicity test containing different soil treatments (CT, MixA or MixC) after 28 days of exposure.

Parameter	Plant part	Lufa	Moisture	F	P	Comparison	P
Length	Shoot	**2.2**	Standard	8.311	0.019	CT vs. MixC	0.018
						CT vs. MixA	0.520
						MixA vs. MixC	0.069
			Flooding	16.412	0.004	CT vs. MixC	0.004
						CT vs. MixA	0.583
						MixA vs. MixC	0.011
		2.1	Standard	10.105	0.012	MixA vs. MixC	0.011
						MixA vs. CT	0.307
						CT vs. MixC	0.068
			Flooding	3.933	0.081	-	-

	Root	**2.2**	Standard	34.925	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	0.958
						MixA vs. MixC	0.001
			Flooding	71.521	<0.001	CT vs. MixC	<0.001
						CT vs. MixA	0.368
						MixA vs. MixC	<0.001
		2.1	Standard	64.767	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	0.101
						CT vs. MixC	<0.001
			Flooding	2.91	0.131	-	-
Dry weight	Shoot	**2.2**	Standard	1.293	0.341	-	-
			Flooding	1.273	0.346	-	-
		2.1	Standard	0.642	0.559	-	-
			Flooding	13.821	0.006	MixC vs. CT	0.005
						MixC vs. MixA	0.222
						MixA vs. CT	0.038
	Root	**2.2**	Standard	2.435	0.168	-	-
			Flooding	15.584	0.004	CT vs. MixC	0.004
						CT vs. MixA	0.072
						MixA vs. MixC	0.069
		2.1	Standard	10.212	0.012	MixA vs. MixC	0.013
						MixA vs. CT	0.750
						CT vs. MixC	0.030
			Flooding	3.038	0.123	-	-

Table S9. Statistical significance of plant-related parameters for *Lolium perenne* (*i.e.*, root and shoot length and dry weight) was assessed in the phytotoxicity test with different soil treatments (CT, MixA, MixC) after 28 days of exposure.

Parameter	**Plant part**	**Lufa**	**Moisture**	**F**	**P**	**Comparison**	**P**
Length	Shoot	**2.2**	Standard	11.67	0.009	CT vs. MixC	0.008
						CT vs. MixA	0.390
						MixA vs. MixC	0.038
			Flooding	0.456	0.654	-	-
		2.1	Standard	2.086	0.205	-	-
			Flooding	0.927	0.446	-	-
	Root	**2.2**	Standard	19.404	0.002	CT vs. MixC	0.003
						CT vs. MixA	0.873
						MixA vs. MixC	0.005
			Flooding	6.984	0.027	MixA vs. MixC	0.033
						MixA vs. CT	0.927
						CT vs. MixC	0.052
		2.1	Standard	53.344	<0.001	MixA vs. MixC	<0.001
						MixA vs. CT	0.087
						CT vs. MixC	<0.001
			Flooding	3.861	0.084	-	-
Dry weight	Shoot	**2.2**	Standard	7.371	0.024	CT vs. MixC	0.021
						CT vs. MixA	0.122
						MixA vs. MixC	0.381
			Flooding	2.01	0.215	-	-

		2.1	Standard	5.835	0.039	CT vs. MixC	0.033
						CT vs. MixA	0.259
						MixA vs. MixC	0.297
			Flooding	3.533	0.097	-	-
	Root	**2.2**	Standard	0.825	0.482	-	-
			Flooding	6.752	0.029	MixC vs. CT	0.026
						MixC vs. MixA	0.123
						MixA vs. CT	0.460
		2.1	Standard	2.865	0.134	-	-
			Flooding	0.742	0.515	-	-

CHAPTER 5

Growth responses of *Trifolium incarnatum* to increasing concentrations of concrete based recycled aggregates in soil

Gaia Mascetti, Roberto Comolli and Chiara Ferré

DISAT-Dipartimento di Scienze dell'Ambiente e della Terra, Università degli Studi di Milano Bicocca, Milano, Italia

In preparation

Abstract

This chapter investigates the responses of *Trifolium incarnatum* to increasing concentrations of concrete-based recycled aggregates (RAs) through a controlled rhizobox experiment.

Plant biomass production and allocation were analyzed to assess plant adaptation strategies. Results showed a clear concentration-dependent responses. Increasing RA contents resulted in a progressive reduction of total plant biomass and significant shifts in biomass allocation between above and belowground organs. These responses suggest adaptive strategies to cope with increased alkalinity and reduced nutrients availability associated with high RA concentrations.

The experiment provides preliminary insights into optimal soil–aggregate proportions aimed at minimizing adverse plant responses while maximizing the potential benefits of RAs in constructed soils.

INTRODUCTION

The production of construction and demolition waste (CDW) during desealing and regeneration interventions represents a major challenge for the sustainability of these projects. As described and documented in previous Chapters of this thesis, recycled aggregates (RAs) generated from these waste have attracted a growing interest not only as secondary raw materials for construction purposes, but also as soil constituent material and growth substrate for sustainable land management and restoration.

When incorporated into soil, RAs can modify its physical characteristics, usually enhancing macroporosity, soil aeration and water flow, but also affect chemical properties such as pH, salinity and nutrient availability [1–4]. However, their impacts on ecosystem functioning, as well as on plant establishment and growth, remain poorly understood.

In previous stages of this research, the influence of RA composition on soil properties and vegetation was investigated, without taking into account different RA concentrations. The desealing field experiment involved the application of a pure layer of RAs (aggregate size 0-4 cm), derived from mixed CDW produced by pavement removal, directly over the desealed soil, demonstrating its potential as growth substrate even without the addition of organic amendments. Conversely, the subsequent indoor mesocosm experiment used a fixed proportion of soil-RA mixture (50% by volume, aggregate size 0-1 cm) and revealed distinct chemical and biological responses depending on RA type, reclaimed asphalt pavement or recycled concrete aggregates.

The results of the mesocosm experiment revealed potential phytotoxic effects of concrete-based RAs particularly on leguminous species, which exhibited a marked inhibition of root growth. Root development in legumes is a key driver of biological nitrogen fixation and overall soil fertility, as the formation of functional nodules and effective nutrient uptake depend on well-developed root systems. However, legumes are also sensitive to nutrient imbalances and elevated pH conditions, commonly induced by the incorporation of concrete based materials into soil, which can result in reduced root development, impaired ion homeostasis, and limited root-bacteria interactions [5].

Plant responses to soil modification and environmental stress are commonly expressed through adaptive strategies which involve changes in biomass allocation, leading to both morphological and physiological adjustments [6]. Under suboptimal or chemically altered conditions, plants may increase resource investment in root rather than shoot growth, or modify the specific root length, to enhance nutrient acquisition efficiency [7]. These adjustments reflect plant adaptation mechanisms and can serve as sensitive indicators of soil health. Therefore, understanding how RA influence root growth and biomass allocation is critical for evaluating the ecological impacts of these materials.

Building on previous findings of this thesis, this experiment investigates the effects of increasing concentrations of concrete based RAs on *Trifolium incarnatum* growth and biomass allocation. The study is conceived as a preliminary experiment toward defining suitable soil-RA proportions for future applications and researches. By identifying concentration thresholds that maintain plant vitality and growth, this work aims to support a safe and sustainable reuse of recycled aggregates in soil systems.

MATERIALS AND METHODS

Rhixoboxes Experimental Design

Two base components were used for use to performed this experiment: uncontaminated agricultural topsoil (0-10 cm) collected from a field in Lodi (Italy), and recycled aggregates obtained from the experimental field at Porto di Mare (Chapter 2). Since these aggregates lack formal certification ensuring their classification as concrete recycled aggregates (RCAs), they are referred to here as concrete based recycled aggregates (RAs).

Prior to the experiment, both soil and RAs were air dried and sieved through 2 mm and 6 mm meshes, respectively. The 0-6 mm represents a common RA size class, also available on the online inert materials market of Lombardy Region [Arpa]. The aggregates were then mixed with the soil to performed five treatments with increasing RA concentrations: 15%, 30%, 45%, 60%, and 75% by weight. To ensure homogeneity, two different RA fractions were incorporated in each treatments according to the original granulometric distribution: 30% in the 2-6 mm fraction and 70% in the 0-2 mm fraction. The experimental design included a negative control consisting of topsoil only.

Specifically designed rhizoboxes (Figure 1), measuring 28 cm in height, 4 cm in width, and 2.5 cm in depth, were realized using PVC cable trunkings. The rhizoboxes were closed at the bottom with cotton wool and filled with approximately 300 g of soil-RA mixture. Ten replicates were prepared for each treatments, including the negative control, and were mounted on panels inclined at 25-30°, to favor a uniform root development [2]. Prior the beginning of the experiments, all the mixtures were irrigated until saturation and allowed to drain for 48 h at ambient temperature [2].

Since previous mesocosm studies suggested that legumes are adversely affected by concrete recycled aggregates, and due to its rapid growth rate and well-developed root system, *Trifolium incarnatum* was the selected species for this experiment. Three seeds were sown in each rhizobox and covered with approximately 1 cm of peat, which helped maintain adequate moisture for seed germination, without significantly altering the chemical properties of the underlying soil-RA mixture. After germination, seedlings were reduced to one plant per rhizobox. Plants were cultivated for two months under controlled conditions (16h: 8h light cycle, 24 ± 2°C air temperature) and regularly watered twice a weeks.

Sampling and Analysis

The chemical characteristic of soil-RAs mixtures were assessed at the beginning of the experiment on the fine fraction (0-2 mm). The pH was potentiometrically measured in a soil-to-water ratio of 1:2.5, total carbonates with the Dietrich-Frühling calcimeter, total carbon and nitrogen with the elemental analyzer (Flash EA 1112 NC-Soil elemental analyzer, Thermo Fisher Scientific CN, Pittsburgh, USA) and available phosphorus through the Olsen method.
The quality of the seeds had been verified through a germination test on filter paper in distilled water (germination rate of 93.3%). Then, for each treatment, the seed germination (SG) of *Trifolium incarnatum* was calculated as the ratio between the number of emerged seedlings and the total number of seeds sown. Plant growth was monitored by direct weekly observations throughout the experiment. After two months, the rhizoboxes were opened using the removable shutter (Figure 1). The shoots were harvested, and roots were carefully sampled and washed with water after their length had been measured. Both roots and shoots were dried at 80°C for 48 hours, and the dry weights of leaves, stems (including branches), and roots were measured for each plant.
The following indices were calculated to assess plant growth strategies and adaptation: specific root length (SRL), calculated as the ratio of roots length to roots dry mass, root to shoot ratio (R:S), and leaves to stems ratio (L:St).
Statistical analyses were performed with R Studio (v. 4.4.2) to evaluate differences in plant parameters among treatments with different RAs concentrations. One-way ANOVA was used when assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) were met. When homogeneity of variance was not satisfied, the non-parametric Kruskal-Wallis test was applied. Post hoc comparison were performed using Tuckey's test.

RESULTS

Soil Properties

The results of chemical analysis on soil-RA mixture are reported in Table 1. The negative control, representing the baseline soil characteristics, exhibited a moderately acidic pH of 5.8, with no detectable carbonates, moderate levels of soil organic carbon (11.7 g/kg) and total nitrogen (1.33 g/kg), and relatively high available phosphorus (61 mg/kg). With increasing RA concentrations, soil pH and carbonate content rose progressively, reaching 9.4 and 44 g/kg in the 75% RA treatment. In contrast, total nitrogen and available phosphorus steadily declined, reaching 0.67 g/kg and 30 mg/kg, respectively, while soil organic carbon (SOC) showed no consistent trend.

T. incarnatum Growth and Biomass

Seed germination of *T. incarnatum* remained high across all treatments, ranging from 90% to 100% (Table 2). Differences among treatments became evident after 3-4 weeks, when plants in the rhizoboxes with 60% and 75% of RAs exhibited reduced growth rates, as evidenced by decreases in height, dimension and number of leaves. After two months, this pattern was confirmed by the quantitative growth measurements.

Roots length was largely unaffected by RA addition, averaging around 26 cm across treatments, with only a slight reduction to 22.7 ± 3.4 cm at 60% RAs. Root dry weight showed a similar trend, with means values around 20 mg and a sharp decline to 4.5 ± 3.3 mg at 60% RAs, and 9.4 ± 1.7 mg at 75% RAs. Up to 45% RAs, the biomass of shoots, leaves, and stems did not significantly differ from the control, although a slight reduction was observed at both 30% and 45% RAs. Higher RA concentrations, however, led to significant growth inhibition in all aerial parts of the plant (e.g. 21.7 ± 4.9 mg of shoots dry weight at 75% RAs, compared to 125.5 ± 54.8 mg in the control).

Specific root length (SRL), which initially averaged around 1.2 cm/mg, strongly increased to 5.04 cm/mg at 60% RAs and remained elevated at 75% RA (2.72 cm/mg), reflecting the strongest decrease of roots dry weight rather than roots length. The root to shoot ratio (R:S) gradually increased with rising RA concentration, from 0.15 in the control to 0.43 at 75% RAs, whereas leaves to stems ratio (L:St) progressively decreased from 1.94 to 1.47 at 75%.

Figure 1. Root system observed after opening the rhizoboxes: (a) in the control and (b) at 60% RAs.

Table 1. Soil chemical properties at different RA concentration.

RAs	pH (H_2O)	$CaCO_3$ (g/Kg)	SOC (g/Kg)	N (g/Kg)	Avail. P (mg/Kg)
0%	5.8	0	11.7	1.33	61
15%	7.2	13	10.8	1.27	57
30%	7.8	21	12.3	1.28	49
45%	8.4	33	12.1	0.94	42
60%	8.8	46	11.7	0.96	38
75%	9.4	44	14.4	0.67	30

Table 2. Measured parameters (mean ± sd) and calculated indices for *T. incarnatum*: roots length (cm); roots, shoots, leaves and stems dry weight (mg); percentage seed germination (SG %); specific root length (SRL; cm/mg); root to shoot ratio (R:S); leaves to stems ration (L:St). Different letters means statistically significant differences in *T. incarnatum* growth parameters among RAs treatments, two months after germination.

RAs	Roots length (cm)		Roots (mg)		Shoots (mg)		Leaves (mg)		Stems (mg)		SG (%)	SRL (cm/mg)	R:S	L:St
0%	25.8 ± 0.6	a	19.1 ± 10	a	125.5 ± 54.8	a	82.8 ± 38.1	a	42.6 ± 16.9	a	93	1.35	0.15	1.94
15%	25.8 ± 0.6	a	21.3 ± 7.8	a	131.0 ± 44.1	a	84.3 ± 28.8	a	46.7 ± 15.7	a	90	1.21	0.16	1.81
30%	26.0 ± 0.0	a	22.9 ± 9.2	a	104.7 ± 42.5	a	76.4 ± 16.5	a	39.9 ± 6.2	a	100	1.14	0.22	1.91
45%	26.0 ± 0.0	a	21.3 ± 4.7	a	103.9 ± 13.1	a	66.4 ± 9.2	a	37.5 ± 5.4	a	93	1.22	0.21	1.77
60%	22.7 ± 3.4	b	4.5 ± 3.3	b	14.6 ± 12.7	b	10.1 ± 7.5	b	6.2 ± 4.9	b	97	5.04	0.31	1.63
75%	25.6 ± 0.8	a	9.4 ± 1.7	a	21.7 ± 4.9	b	12.9 ± 3.2	b	8.8 ± 2.0	b	93	2.72	0.43	1.47

DISCUSSION

Soil Properties

Negative control characteristics are consistent with a fertile, nutrient-rich, moderately acidic agricultural topsoil and provide a baseline for assessing the effects of RAs addition.

The increase of pH and carbonate content with increasing RA concentrations confirms the alkalinizing effect of the concrete based RAs, consistent with their mineral composition. However, the high pH values observed at the highest RA concentration (up to 9.4 at 75% RAs) are unlikely to be explained by carbonates alone and may also reflect the presence of soluble alkali metal oxides, such as Na_2O and K_2O, commonly associated with cementitious materials, which can release sodium and potassium ions in soil [8].

In contrast, total nitrogen and available phosphorus decreased progressively, likely due to dilution effect resulting from the higher proportion of RAs relative to soil.

Soil organic carbon showed no clear trend as effect of RAs addition, suggesting that RAs may contain a small amounts of organic matter. This organic component is likely originated from soil material incidentally incorporated into demolition waste during pavement removal [9]. However, the concurrent decrease in total N at higher RA contents suggests that such organic matter, if present, is characterized by a high C:N ratio, resulting in a negligible contribution to the soil nitrogen pool.

T. incarnatum Responses

The results indicate that RA addition did not impaired *T. incarnatum* germination rate, which remained consistently high across all treatments. However, plant growth and biomass allocation were significantly affected by high RA concentrations.

According to its ecological preferences [10,11], *T. incarnatum* thrives in slightly acidic to neutral soils (pH 4.5–7.5), nutrient-rich and moderately moist. Thus, the progressive increase in pH observed in the mixtures with higher RA concentrations created conditions that deviate from the species' optimal range. Elevated pH levels may alter root membrane permeability and ion exchange capacity, restricting nutrient uptake and exacerbating stress responses [12]. Furthermore, alkaline conditions tends to decrease the mobility and availability of key nutrients, with the precipitation of phosphates and metal ions [13], that may results into inadequate concentrations for plant growth.

In contrast, *T. incarnatum* shows a certain tolerance to salinity [11], that likely increased with rising RA concentration, as demonstrated in the previous indoor-mesocosm experiment (Chapter 3) due to salts release from concrete based RAs.

While root length was largely unaffected by increasing RA concentrations, root biomass showed a pronounced reduction, leading to a marked increase in specific root length. This morphological adjustment suggests a shift in root architecture favoring elongation over biomass accumulation. Such increase in SRL index is commonly associated with an adaptive strategy, aimed at enhancing the root system's capacity to explore soil and acquire nutrients [14,15]. Indeed, in nutrient poor soils or under stressful conditions, allocating a greater proportion of biomass to roots enables plants to maintain nutrient uptake efficiency, support essential metabolic functions, and increase survival chances [6].

The significant reduction in shoot biomass at higher RA concentrations reflects a clear inhibitory effect on aboveground growth, that affected both leaf expansion and stem development. The gradual increase in root to shoot ratio (R:S) with rising RA concentrations indicates that plants reallocates biomass preferentially to roots under higher RA exposure. This trend further confirm the plant adaptive strategy aimed at optimize nutrient uptake, by investing more resources into root development [7]. The progressive decrease in leaf to stem ratio (L:St) indicates a different biomass allocation also among the aerial parts of the plant, prioritizing stems development, which provide structural support and transport functions, rather than leaves, thereby reducing investment in photosynthetic capacity [16].

CONCLUSION

The experiment with *Trifolium incarnatum* demonstrated that the addition of concrete based RAs in soil significantly affected soil chemical properties, plant growth and biomass allocation patterns.

The progressive increase in pH and carbonate content confirmed the alkalinizing effect of RAs, while the decline in total nitrogen and available phosphorus, likely due to the dilution effect of higher soil-RA ratios, indicated a reduction in soil fertility with higher aggregate concentrations. Although seed germination was not impaired, vegetative growth and total biomass were substantially reduced at high RA levels (≥60%), suggesting that the species can only partially tolerate such conditions.

The observed morphological responses, particularly the increase in the root to shoot ratio and specific root length, reflect adaptive strategies typically associated with nutrients depletion and chemical stress. These adjustments indicate that plants attempted to compensate the adverse conditions by allocating more resources to root development, enhancing nutrient acquisition potential, rather than shoot growth. Moreover, the leaf to stem decrease indicates a further adaptation that prioritize structural support rather than photosynthetic activity. These shifts in resource allocation increase the survival chances of the plants, but they concurrently lead to an overall decline in physiological performance and productivity.

Overall, the experiment provides valuable insights on the potential effects of the fine fraction of concrete based RAs on a leguminous species, and highlights the need for caution when incorporating them into soil. Moderate RA concentrations (≤45%) may represent a suitable compromise between promoting sustainable material reuse, for example after desealing interventions, and maintaining an adequate ecological functionality of soil.

Further studies are essential to improve our understanding of RA effects on plant growth dynamics, and should investigate, for example, different plant species, more finely spaced RA concentration intervals, and the possible presence of allelopathic compounds or other inhibitory elements within RAs.

Bibliography

1. Mastrolando, G.; Giagnoni, L.; Maienza, A.; Fantoni, G.; Rompato, B.; Argenti, G.; Selvi, F.; Fiore, B.; Certini, G. Use of Waste Materials as Feasible Strategy for Urban De-Sealed Soil Reclamation: Evidence from a Restoration Case Study in Prato, Italy. In Proceedings of the 13th International Symposium of the International Union of Soil Sciences on Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA 13); 2025; pp. 158–159.
2. Coull, M.; Butler, B.; Hough, R.; Beesley, L. A Geochemical and Agronomic Evaluation of Technosols Made from Construction and Demolition Fines Mixed with Green Waste Compost. *Agronomy* 2021, *11*, doi:10.3390/agronomy11040649.
3. Mikajlo, I.; Pando, A.; Robain, H.; Lerch, T.Z. Reusing Asphalt Millings with Excavated Materials and Compost to Construct Technosols: Effects on Soil Properties and Plant Growth. *J Soils Sediments* 2024, doi:10.1007/s11368-024-03942-3.
4. Herbreteaua, A.; Schwartzb, C.; Beguiristaina, T.; Bojicc, C.; Cossu-Leguillec, C.; Durandb, A.; Leglizeb, P.; Lorgeouxd, C.; Faurea, P. Reactivity of Road Asphalt Buried in Soils after De-Sealing Operations. In Proceedings of the 13th International Symposium of the International Union of Soil Sciences on Soils in Urban, Industrial, Traffic, Mining and Military Areas (SUITMA 13); 2025; pp. 205–206.
5. Kumar, K.; Jaiswal, A.; Koppolu, U.M.K.; Kumar, K.R.R. Alkaline Stress Disrupts Growth, Biochemistry, and Ion Homeostasis of Chickpea (Cicer Arietinum L.) Roots. *Frontiers in Agronomy* 2024, *6*, doi:10.3389/fagro.2024.1497054.
6. Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass Allocation to Leaves, Stems and Roots: Meta-analyses of Interspecific Variation and Environmental Control. *New Phytologist* 2012, *193*, 30–50, doi:10.1111/j.1469-8137.2011.03952.x.
7. Lopez, G.; Ahmadi, S.H.; Amelung, W.; Athmann, M.; Ewert, F.; Gaiser, T.; Gocke, M.I.; Kautz, T.; Postma, J.; Rachmilevitch, S.; et al. Nutrient Deficiency Effects on Root Architecture and Root-to-Shoot Ratio in Arable Crops. *Front Plant Sci* 2023, *13*, doi:10.3389/fpls.2022.1067498.

8. Plusquellec, G.; Geiker, M.R.; Lindgård, J.; Duchesne, J.; Fournier, B.; De Weerdt, K. Determination of the PH and the Free Alkali Metal Content in the Pore Solution of Concrete: Review and Experimental Comparison. *Cem Concr Res* 2017, *96*, 13–26, doi:10.1016/j.cemconres.2017.03.002.
9. Page, J.L.; Winston, R.J.; Hunt III, W.F. Soils beneath Suspended Pavements: An Opportunity for Stormwater Control and Treatment. *Ecol Eng* 2015, *82*, 40–48, doi:10.1016/j.ecoleng.2015.04.060.
10. Landolt, E.; B.B.; E.A.; H.O.; K.F.; L.W.; N.M.; R.-M.K.; S.F.H.; T.J.-P.; U.E.; V.M.; W.T. *Flora Indicativa – Ökologische Zeigerwerte Und Biologische Kennzeichen Zur Flora Der Schweiz Und Der Alpen*; Haupt Verlag, 2010;
11. InfoFlora Trifolium Incarnatum L. Subsp. Incarnatum – Trifoglio Incarnato Available online: https://www.infoflora.ch/it/flora/trifolium-incarnatum-subsp-incarnatum.html (accessed on 31 October 2025).
12. Yang, S.; Xu, Y.; Tang, Z.; Jin, S.; Yang, S. The Impact of Alkaline Stress on Plant Growth and Its Alkaline Resistance Mechanisms. *Int J Mol Sci* 2024, *25*, 13719, doi:10.3390/ijms252413719.
13. Penn, C.; Camberato, J. A Critical Review on Soil Chemical Processes That Control How Soil PH Affects Phosphorus Availability to Plants. *Agriculture* 2019, *9*, 120, doi:10.3390/agriculture9060120.
14. Ostonen, I.; Püttsepp, Ü.; Biel, C.; Alberton, O.; Bakker, M.R.; Lõhmus, K.; Majdi, H.; Metcalfe, D.; Olsthoorn, A.F.M.; Pronk, A.; et al. Specific Root Length as an Indicator of Environmental Change. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* 2007, *141*, 426–442, doi:10.1080/11263500701626069.
15. Keerthi, G.M.; Mallikarjuna, M.G.; Jha, S.K.; Pandey, R.; Veeraya, P.; Lohithaswa, H.C.; Chinnusamy, V. Unravelling Root System Architecture Plasticity in Response to Abiotic Stresses in Maize. *Sci Rep* 2025, *15*, 20433, doi:10.1038/s41598-025-04123-z.
16. Lloveras, J.; Iglesias, I. Morphological Development and Forage Quality Changes in Crimson Clover (*Trifolium Incarnatum* L.). *Grass and Forage Science* 2001, *56*, 395–404, doi:10.1046/j.1365-2494.2001.00289.x.

CHAPTER 6

General Conclusions

GENERAL CONCLUSIONS

Desealing and soil reconstruction using recycled materials represent innovative approaches for restoring soil functionality and promoting sustainable urban regeneration. By integrating field investigations with controlled laboratory experiments covering microbiological, chemical, phytotoxic, and climatic dimensions, this thesis advanced the understanding of desealed soil recovery dynamics and of the potential use of recycled aggregates (RAs) as soil constituent materials in urban environments.

Soil recovery after desealing

The field experiment at Porto di Mare revealed that the restoration of soil functionality after desealing occurs through distinct processes operating at different temporal scales. The removal of impervious surface resulted in an immediate improvement of soil pedoclimatic conditions, including enhanced aeration, water infiltration and retention, and temperature regulation. These physical changes created favorable conditions for microbial activity and biological processes, indicating that desealing can promptly reactivate key soil functions even in highly disturbed urban soils. In contrast, soil chemical properties (*i.e.*, organic carbon and total nitrogen content) evolved more gradually, showing almost imperceptible changes within the one-year monitoring period. Compost addition and vegetation sowing induced only limited and temporary changes in soil properties and microbial community, whereas temporal fluctuations were mainly associated with seasonal dynamics of pedoclimatic conditions and organic inputs.

Overall, these results highlight the importance of considering soil restoration as a progressive process, in which early pedoclimatic and biological improvements may precede and potentially drive longer-term improvements in soil organic matter and associated ecosystem services, including carbon storage.

Environmental suitability of RAs under field conditions

The application of RAs from pavement demolition waste did not result in harmful leaching of heavy metals into percolation water, confirming their environmental safety and suitability as soil constituent materials when properly selected and characterized. Moreover, RAs provided a suitable growth substrate, not limiting the establishment and the development of different vegetation type. Although the short duration of the field experiment limits the assessment of long-term trends, these results suggest that recycled aggregates can be effectively integrated into desealing practices without compromising soil quality, while organic amendments and vegetation are expected to progressively promote soil chemical and biological functions over longer timescales.

Soil and plant responses to RAs under controlled conditions

The indoor experiments conducted under controlled conditions provided essential insights into the mechanisms and potential constraints associated with the application of RAs on soil systems and plant responses.

The mesocosm experiment using the fine fractions (0-1 cm) of recycled concrete aggregates (RCA) and reclaimed asphalt pavement (RAP), mixed with soil in a concentration of 50% by volume, demonstrated that both materials substantially modified soil physicochemical properties, microbial activities, and early-stage plant development. While physical changes generally improved water drainage and soil compaction, increases in pH and electrical conductivity strongly affected nutrient availability and microbial processes, especially in soil-RCA mixture. The experiment also evidenced that magnitude of these effects strongly depends by initial soil properties, moisture regime, plant species.

Consistent and complementary results were provided by the rhizobox experiment, that assesses the effects of a finer fraction (0-6 mm) of concrete-based RAs on *Trifolium incarnatum* growth. Increasing RA concentrations resulted in reduced nutrient availability and increased soil pH, which in turn led to lower biomass production and morphological adjustments indicative of adaptive strategies and shifts in resource allocation during early to intermediate vegetative stages. Under these conditions, a threshold concentration of approximately 45% RA fine fraction was identified as the upper limit for maintaining soil functionality and supporting plant performance. However, potential negative effects on different plant species or over longer growth periods (e.g. during reproductive stage) cannot be excluded and require further investigation.

Overall, laboratory findings revealed that the composition, particle size, and concentration of RAs in soil critically determine their ecological impact, which may also varies according environmental conditions and initial soil fertility.

Implications for urban soil restoration and future perspectives

The results of this thesis demonstrate that soil desealing can effectively initiate soil recovery in urban environments and that RAs can be incorporated into soil reconstruction practices when their properties and application conditions are carefully managed. At the same time, the findings highlight the need for caution in using high concentrations of fine RAs fractions, which may adversely affect soil chemical balance, microbial activity, and plant development.

Future research should prioritize long-term monitoring of desealed sites to evaluate the persistence and stability of restored soil functions across multiple years. Further investigations are also required to better understand the degradation pathways, leaching behavior, and potential toxicological effects of recycled materials in reconstructed soils under varying environmental conditions, including the role of emerging contaminants. Ultimately, advancing knowledge in this field should support the development of operational, site-specific guidelines for urban soil reconstruction and restoration, contributing to more sustainable and resilient cities.

Acknowledgements

I would like to express my sincere gratitude to my supervisor, **Dr. Chiara Ferré**, for her guidance, support, and constant availability throughout my doctoral journey. I am also deeply grateful to **Professor Roberto Comolli** for his valuable advice and constructive discussions during both the experimental and interpretative phases of this research. I would also like to thank **Professor Antonio Finizio** for his role as my PhD tutor and for his institutional support during my doctoral program.

A special acknowledge goes to my tutors abroad, **Dr. Sara Peixoto** and **Professor Susana Loureiro**, for welcoming me at the University of Aveiro and for their scientific guidance, availability, and support during throughout my research stay.

I would also like to thank **Professor Isabella Gandolfi**, **Dr. Francesca Pittino**, and the PhD student **Isabella Ghizzi** for their support and expertise in microbiological analyses, as well as for the insightful discussions that enriched the interpretation of the results.

My sincere thanks go to the laboratory technician **Fabio Moia,** as well as to the PhD students of the geopedology research group **Davide Abu El Khair**, **Camilla De Feudis**, and **Sara Agaba**, for their invaluable help during fieldwork and laboratory activities. I am also grateful to all the PhD students with whom I shared this journey, for the stimulating discussions, collaboration, and mutual support.

Finally, I would like to thank all those who supported me outside the academic environment, for their patience, understanding, and encouragement throughout these years. Their presence and support made this journey possible and meaningful.