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# Long-term field evaluation of sewage sludge biochar in green roof substrates reveals hydrological, vegetation, and microbial responses

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## Abstract

Sewage sludge biochar (SSB) represents a circular material with the potential to improve the ecological performance of extensive green roofs and valorize nutrient-rich waste streams. Here, we report a 40-month rooftop monitoring experiment under temperate conditions evaluating the effects of SSB incorporation (10% and 20% v/v) on substrate properties, hydrological performance, runoff quality, vegetation development, and microbial diversity. SSB amendment reduced bulk density and increased total porosity and water-holding capacity, resulting in higher substrate moisture availability. These changes led to improved water retention, with annual runoff reduced by 3.3–8.5%, with the highest reduction observed in the SB20 treatment during the driest year (2022), and cumulative seasonal retention reaching 76% during dry years. Despite the nutrient-rich origin of SSB, runoff nutrient concentrations exhibited initial increases and subsequent stabilization, consistent with gradual nutrient release dynamics. Vegetation cover reached to 73% in the control and to 84% (SB20) and 89% (SB10) over the study period, with no significant difference between the two amendment rates. Microbial analyses revealed higher bacterial and fungal richness in SSB treatments, particularly at 20% v/v. Structural equation modelling indicated that variation in microbial richness was associated with changes in runoff chemistry (pH, electric conductivity, total suspended solids) and interannual dynamics, rather than effects explained by a single factor. Overall, this study provides evidence from extended rooftop monitoring, highlighting long-term system responses of extensive green roofs amended with SSB, particularly regarding water balance, substrate physical development, and microbial diversity.

## Keywords

sewage sludge biochar, extensive green roof substrate, water management, runoff quality, vegetation, microbial diversity

## Introduction

As a component of green infrastructure, green roofs deliver both direct and indirect benefits and can help reduce the adverse effects of urbanization, such as the heat island effect, urban flooding, and air and water pollution[1]. Green infrastructures are highlighted in the EU's climate change adaptation strategy as versatile, no-regret interventions that simultaneously offer environmental, economic, and social advantages and strengthen resilience to climate impacts[2]. The substrate composition and quality strongly influence green roof ecological functions because the substrate, as the most important layer, supports plant growth and provides a habitat for microorganisms[3], and guarantees stormwater management performance[4]. However, there is no universally defined composition of the roofing substrate. Due to the use of large quantities of primary minerals (spongilite, pumice), non-renewable or energy-demanding materials (peat, expanded clay) when preparing especially extensive roof substrates, green roofs can have a negative environmental impact[5]. The extraction and processing of these components contribute to the depletion of non-renewable resources, can generate substantial greenhouse gas emissions, and require significant energy input. In addition to these material-related impacts, green roofs may also pose urban environmental risks through the chemical leaching of nutrients[6], heavy metals[7], and micropollutants originating from construction materials[8]. As reviewed by Farah et al.[9], several recent studies have focused on integrating local materials (increasing the sustainability), recycled materials (supporting a circular economy), and high-performance materials into substrates to mitigate these adverse effects.

Biochar, obtained through the pyrolysis of organic materials has recently emerged as a promising soil-less component for green roof installations (see review by Lee and Kwon[10]). Its application improves water holding capacity[11,12] as well as the quality of runoff [13], improves substrate structure[14], plant nutrient status and availability, and influences microbial community by modulating substrate moisture levels.

Prior research has primarily focused on plant-based biochar, whose effectiveness is influenced by the feedstock biomass, pyrolysis parameters, and application rate. However, the use of sewage sludge biochar (SSB) with high circular economy potential in roofing substrates remains poorly characterized, as only a limited number of studies have investigated this area of research[3,5,15,16]. Sewage sludge (SS) is an abundant, nutrient-rich waste stream (phosphorus (P), nitrogen (N), micronutrients) that is often underutilized. The reason is that in several EU countries, its direct agricultural use is not recommended due to the presence of heavy metals, micropollutants, and pathogens[17]. As stated in the European Biochar Industry Consortium position paper[18], the pyrolysis of SS enables removal of the most important contaminants of high ecological impact and production of a valuable phosphate fertilizer in the form of sewage sludge biochar, and, within that policy framework, has been described as having a net positive impact on the climate. Given the stressful environment of green roofs, SSB may be an essential amendment; however, there is a lack of comprehensive information about the long-term behavior of SSB in roofing substrates. Chen et al.[3] demonstrated that sludge biochar application (10 and 15% v/v) increased substrate moisture, reduced temperature, and increased plant biomass and microbial abundance under short-term experimental conditions. However, their study was limited to a single growing season and did not address long-term hydrological performance or system responses under prolonged rooftop exposure. Moreover, concerns remain about potential heavy metal leaching or nutrient release dynamics. Only a limited number of research works have examined the effect of SSB on both the quantity and quality of green roof runoff. Vavrincová et al.[16] analyzed discharge chemistry and seasonal heavy metal dynamics during the first 30 months of monitoring using the same rooftop experimental system. They demonstrated that the application of SSB did not increase the risk of runoff water contamination relative to a commercial roofing substrate and showed, based on the analysis of heavy metals in the above-ground parts of *Sedum* plants, that SS pyrolysis significantly reduces metal bioavailability. That study focused primarily on runoff quality and the risks associated with heavy metal mobility, whereas the broader long-term system evaluation, including hydrological performance, substrate aging processes, vegetation cover dynamics, and microbial richness patterns, remained outside its main focus. Novotný et al.[19] confirmed that SSB application (8.2% v/v) to roofing substrate results in a minor increase in the leaching of nitrogen, phosphorus, and heavy metals (Cu, Hg, Zn). Nonetheless, it remains unclear whether incorporating SSB into roofing substrates provides long-term improvements in water management, a benefit that has been demonstrated in the case of plant-based biochar applications[12,20].

In view of the above, this study extends previous findings by broadening the analytical scope toward a long-term, system evaluation that integrates hydrological performance, substrate aging processes, vegetation cover dynamics, and microbial richness under real rooftop conditions. Specifically, this research aimed to: (i) quantify how SSB alters the physical, chemical, and hydraulic properties of virgin and aged substrates, (ii) evaluate its long-term effects on water balance, rainfall retention, and runoff generation under natural rooftop conditions, (iii) assess the nutrient leaching risks and runoff quality implications of SSB over multiple years, and (iv) determine how SSB influences plant cover dynamics and microbial diversity.

## Material and methods

### *Preparation and characterization of sewage sludge biochar and roofing substrates*

Sewage sludge biochar (SSB) was generated using sludge from a municipal wastewater treatment plant by a continuous slow-pyrolysis unit (Pyreka 2.1, Pyreg GmbH, Germany) operated at 600 °C with an average residence time of approximately 10 min. The resulting SSB was thoroughly analyzed following standard analytical procedures (see Supplementary materials 1 for details). The material consisted predominantly of particles < 10 mm and was used as a green roof substrate amendment without further size fractionation.



A commercial extensive green roof substrate from a local supplier (JV Intersad, Slovakia) served as the base material. The base substrate was characterized by a slightly alkaline pH (7.42), dry bulk density (DBD) of 1.04 g $cm^{-3}$, total porosity (TPo) of 43%, and a water holding capacity (WHC) of 38.6%. Total organic carbon (TOC) was 0.86 weight %, total nitrogen (TN) 0.1 weight % and total phosphorus (TP) 83.8 mg $kg^{-1}$. Detailed physical and chemical characteristics are provided in Table 2. Two experimental substrates were prepared by volumetric incorporation of SSB at 10% (SB10) and 20% (SB20) and homogeneously mixed, while the unamended substrate served as the control (SB0). Composition of tested roofing substrates is shown in Table 1 and Fig. S2.

The physical characteristics of the homogenized extensive roofing media were analyzed in triplicate. Measurements included hydraulic conductivity ($K_f$), water holding capacity (WHC), saturated bulk density (SBD), dry bulk density (DBD), and organic matter (OM) content, all carried out in accordance with the National Standards for Vegetated Roofs[21] and the FLL Green Roof Guidelines[22]. Total porosity (TPo) and air-filled porosity (AFP) were assessed following the method outlined by Latshaw et al.[23]. The particle size distribution was assessed by subjecting oven-dried substrate (500 g, 48h at 50°C) to a series of sieves of various apertures using an analytical sieve shaker (CISA RP200 N). Water retention curves were obtained using a pressure plate apparatus (Soil Moisture Equipment Corp., USA) across nine matric potentials (0–1500 kPa), and fitted using the van Genuchten model[24]. More details are shown in Supplementary materials 2.

Table 1. Composition (percentage by fresh volume) of prepared extensive roof substrate.

| **Component** | % | | |
|---|---|---|---|
| | **SB0** | **SB10** | **SB20** |
| Sewage sludge biochar | 0 | 10 | 20 |
| Compost | 20 | 18 | 16 |
| Recycled bricks (2–12 mm) | 30 | 27 | 24 |
| Recycled bricks (6–20 mm) | 30 | 27 | 24 |
| Rhyolite | 10 | 9 | 8 |
| Expanded clay | 10 | 9 | 8 |

Substrate pH, electric conductivity (EC), and total dissolved solids (TDS) were measured in 1:5 (w/v) extracts (deionized water for pH, 0.01 M $CaCl_2$ for EC and TDS) applying a HI5521 pH/EC/TDS meter (Hanna Instruments, EU). Concentrations of As, Cd, Cr, Co, Cu, Hg, Ni, Pb, Se, and Zn in the prepared substrates were quantified following wet digestion with $HNO_3$ and $H_2O_2$ using an Agilent 200 Series atomic absorption spectrometer (AAS) with SpectrAA Software (Agilent, USA) after wet digestion with $HNO_3$ and $H_2O_2$.

### *Experimental green roof setup*

A long-term rooftop study was launched in October 2020 on the Faculty of Education building at the Trnava University, Slovak Republic (N48°21'43.9", E17°35'48.5"; 150 m a.s.l.), and was continuously monitored for 40 months. The Trnava region is among the hotest areas of Slovakia with an average annual temperature of 9 - 10°C. The long-term average annual rainfall is 560 mm, and overall the area is one of the rainfall-deficient regions. Among the local climatic factors, wind shows the greatest variability, with northwestern (NW) winds prevailing across all seasons.

The study comprised 12 rooftop experimental platforms (dimensions: 0.6 m × 0.4 m; depth: 0.12 m), each equipped with an independent drainage outlet (diameter 0.5 cm) at the base connected to a separate runoff collection canister. The units were elevated 0.4 m above the rooftop, and inclined at a slope of 4.5° to simulate extensive green roof conditions (Fig. 1). The experimental design included three extensive substrate variants, each replicated four times: a control substrate without sewage sludge biochar (SB0), and two SSB-amended substrates containing 10% and 20% SSB by volume. The 12 units were arranged in three treatment groups (SB0, SB10, SB20; Fig. 1), each consisting of four physically separated plots elevated above the rooftop surface. Although all units were located on the same rooftop platform, runoff was collected and analyzed separately for each plot, and statistical analyses were conducted using individual plots as experimental replicates (n = 4 per treatment). In each experimental plot, the layering structure of an extensive green roof system was simulated. At the base of each unit, a Nophadrain ND100 drainage layer (0.8 mm) equipped with a pressure-dividing slip film and a filter geotextile was installed. Subsequently, 16 L of the prepared substrate mixtures (SB0, SB10, SB20) were applied as an 80 mm layer and gently compacted to achieve uniform density. In each unit, 9 *Sedum* plug plants (*S. reflexum, S. spurium, S. sexangulare, S. spurium voodoo*) were planted with 15 cm distances between individual plants. The selected *Sedum* species are widely applied in commercial extensive green roofs in Central Europe due to their drought tolerance. During installation, uneven rainfall exposure occurred due to the sequential placement of the experimental units under rainy conditions. To partially compensate this imbalance, additional collected rainwater was added (10 mm to SB10 plots and 30 mm to SB20 plots). Complete equalization was not feasible due to the magnitude of the rainfall event. These supplementary inputs were included in the precipitation totals used for the subsequent runoff calculations. The experimental green roof installation and long-term monitoring were conducted with formal permission from the Faculty of Education, Trnava University in Trnava (Slovak Republic), the owner and administrator of the building.




Fig. 1. Experimental setup installed on the rooftop of the Faculty of Education (Trnava University in Trnava, Slovak Republic). Plots contain roofing substrates without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v). S – moisture/temperature sensor; D – datalogger; E – canister for runoff collection; WL – weighing lysimeter setup; WS – weather station.

### *Monitoring of microclimate, runoff, and evapotranspiration*

Substrate temperature and volumetric water content (moisture) were continuously monitored in the experimental plots at 30-minute intervals using SMT100 sensors (Truebner, Germany), placed in the centre of the plots at a 3 cm depth. Data were collected on a TruLog 100 datalogger using TrueLog 100-configuration software V2.5.0 (Truebner, Germany). Since July 2021, monitoring of the small-scale lysimeter setup has started. A weighing lysimeter with a 50 kg load cell and 0.01 kg resolution (Zemic 6LG) was located under the three experimental units (Fig. 3, dimensions: 0.6 m × 0.4 m; depth: 0.12 m), and the weight of the boxes was recorded at 15-minute intervals. The collected data are available via an application developed in cooperation with DomAtom, Ltd. (Slovakia) for this purpose. A funnel was placed at the draining end of each experimental unit to capture the discharge into a plastic canister. Runoff volumes expressed as equivalent water depths (mm) were determined only following precipitation events that generated measurable discharge (predominantly moderate and heavy rainfall), except during the summer of 2023, when the amount of captured runoff was measured in September 10. Seasonal and annual runoff reduction (%) was calculated as:

$$\text{Runoff reduction} = \left(1 - \frac{\text{cumulative runoff depth}}{\text{cumulative precipitation}}\right) \times 100) \quad (1)$$

where cumulative precipitation (mm) and runoff depths (mm) were determined for the respective seasonal or annual period. Antecedent substrate moisture conditions were not parameterized but are reflected in the cumulative runoff response over the defined periods.

Runoff samples were collected for water quality analysis, measuring EC, TDS, and pH with HI5521 meter, chemical oxygen demand (COD) by the potassium dichromate oxidation method, and total suspended solids (TSS) using TN400 turbidimeter (Apera Instruments). Samples for TP and TN analysis were oxidized using Spectroquant Crack set 10 or 20 (Merck, Germany) in a thermoreactor (1 h, 120°C) before spectrophotometric measurements using Spectroquant – phosphate and nitrate tests (Merck, Germany). Runoff chemistry data for the first 30 months partially overlap with those reported in Vavrincová et al.[16] However, the present analysis is restricted to aggregated interannual summaries and incorporates an additional 10 months of monitoring.

Reference evapotranspiration ($ET_0$, mm day$^{-1}$) for the green roof experimental units was derived from climatic data using the Penman–Monteith equation (2), following the approach of Zotarelli et al.[25]:

$$ET_0 = \frac{0{,}408\Delta(R_n - G) + \gamma \dfrac{\gamma 900}{T + 273} u_2(e_s - e_a)}{\Delta + \gamma(1 + 0{,}34u_2)} \quad (2)$$

where $T$ is the daily average air temperature recorded 2 m above the rooftop (°C), $u_2$ is wind speed (m $s^{-1}$) at 2 m above the rooftop and $\gamma$ is the psychrometric constant (kPa $°C^{-1}$). G represents heat flux density and $R_n$ is net radiation, and both are expressed in MJ/$m^2$/day. $\Delta$ is the slope of the vapor pressure curve (kPa $°C^{-1}$) and $e_a$ and $e_s$ are actual and saturation water vapor pressure (kPa).

Actual evapotranspiration ($ET_{lys}$) was estimated through mass balance analysis of weighing lysimeter records:

$$ET_{lys} = P - \Delta W - R \quad (3)$$

where $P$ is precipitation (mm/d), $\Delta W$ denotes the daily lysimeter weight change, calculated as $W_0 - W_{24}$, with $W_0$ and $W_{24}$ representing weights at 00:00 and 24:00, respectively, and $R$ is the runoff from experimental plots (mm $d^{-1}$). Weight changes were converted to mm of water. Meteorological data (air temperature, atmospheric pressure, relative humidity, precipitation, and wind velocity) were recorded using a meteorological station (Garni 935 PC, Czech Republic) positioned 2 m above the rooftop adjacent to the experimental units.

### *Vegetation cover*

Plant coverage and *Sedum* species abundance were evaluated repeatedly during the monitoring period (March 2021; May 2021; September 2021; February 2022; May 2022; June 2022; September 2022; October 2022; April 2023; May 2023; and October 2023). The assessment dates were selected to capture seasonal and interannual changes in plant cover. Substrate cover by *Sedum* plants in each experimental units was determined by digital image analysis. The images were analyzed using the ImageJ program[26], with Color Threshold (CT) technique, in which the hue, saturation, and brightness were adjusted according to the background characteristics of each plot. The same thresholding procedure was applied across all sampling dates to ensure comparability. The result of the analysis was a binary image where black pixels correspond to the selection criteria – *Sedum* plants. Although a formal classification accuracy assessment was not performed within this study, the CT method has been shown to provide reliable estimates of vegetation cover on extensive green roofs, demonstrating strong agreement with manual segmentation[27].



### *Microbial genetic diversity*

Roof substrate samples from 4-5 cm depth were collected at the ends of May 2021 and 2022 from all experimental plots (SB0, SB10, and SB20). Twenty-four samples were collected (12 in 2021 and 12 in 2022), with substrate taken from three different locations within one box and mixed into a single sample in a 50 mL sterile tube. Total/metagenomic DNA was extracted from roof substrate samples using the DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's protocol. DNA quantity and purity were measured spectrophotometrically (NanoDrop-1000, Thermo Scientific Inc.) and diluted to a final concentration of 25 ng $\mu L^{-1}$. Bacterial and fungal genetic diversity was identified applying automated ribosomal intergenic spacer analysis (ARISA) as a community fingerprinting approach for comparing relative richness and community structure among treatments, following the method outlined by Ondreičková et al.[28].

### *Statistical analyses*

The effects of substrate age and substrate treatment (SB0, SB10, SB20) on the physical and chemical properties were evaluated separately using one-way ANOVA. When significant differences were detected, Tukey's Honestly Significant Difference (HSD) post hoc test was applied. Statistical significance was set at $p \leq 0.05$.

Statistical evaluations of bacterial and fungal diversity obtained from ARISA profiles were performed using ANOVA with a 95% confidence level. Where significant differences were detected ($p \leq 0.05$), Least significant difference (LSD) *post hoc* tests were implemented using Statgraphics XVII-X64 (Statpoint Technologies, Inc.). Alpha diversity indices (Simpson, Shannon, Evenness) were computed in PAST v3.19[29]. UpSet plots were generated using the UpSetR Shiny an online tool accessible at https://gehlenborglab.shinyapps.io/upsetr/. Intersection sizes and total set sizes were used to visually compare operational taxonomic unit (OTU) overlap and richness distribution across all experimental combinations. The UpSet technique and web tool were used as described by Lex et al.[30].

Structural equation modelling (SEM) was employed to integrate the effects of SSB and year on runoff characteristics and biological responses beyond univariate analyses. This was done using the PATHj module[31] in jamovi (version 2.6)[32], based on the *lavaan* package in R[33]. SSB application rate and year were treated as an exogenous variable, while runoff quality parameters and biological indicators were included as endogenous variables to evaluate direct and indirect associations of SSB and year effects. To aid in the interpretation and visualization of model relationships, semPlot[34] was used for path diagram generation. Data were standardized using Z-score transformation before analysis. Model parameters were estimated using the maximum likelihood estimator (MLM).

## Results and discussion

### Green roof substrate properties

In our experiments, the incorporation of SSB resulted in distinct changes in the physical and chemical properties of unused virgin substrates and 40-month-old samples from experimental green plots (Table 2).

***Virgin substrates*.** As expected, SBD and DBD decreased moderately by 2.1 – 5.1% and 8.3 – 9.1%, respectively, after applying SS biochar. On the contrary, the measured WHC values were 41.6% for SB10 and 44.0% for

SB20, representing increases of 7.7% and 14.0%, respectively, compared to the control. Both values remained within the limits recommended by the FLL guidelines for extensive green roofs[22]. Similarly, TPo increased to 46.2% for SB10 and 50.4% for SB20, corresponding to increases of 7.4% and 17.2% (Table 2). Although most studies report WHC improvements in roofing substrates amended with plant-based biochars[12,35,36], which differ markedly from SSB in their physical and chemical properties, the present results demonstrate that SSB is similarly effective in increasing substrate water retention. This response is consistent with previous studies using biochar from different feedstocks in extensive green roof systems [3,37]. The water retention curves (WRCs, Fig. S3) indicated a rightward shift in the substrates amended with biochar, particularly evident in the pressure range of 0-100 kPa (pF points 1-3), where higher moisture contents were measured compared to the SB0. This finding aligns with previous research on biochar-amended green roof substrates[20,35], supporting the conclusion that biochar increases the substrate's water retention at low to intermediate suction pressure heads.

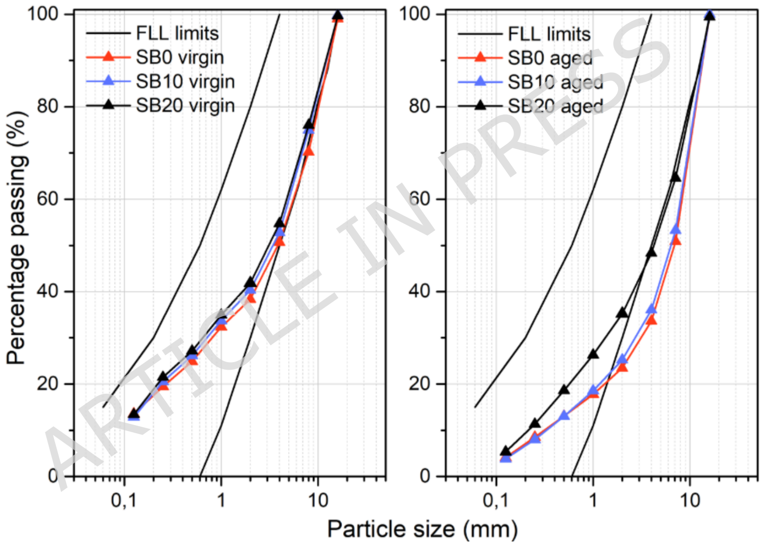


Fig. 2. Particle size distribution curves of both virgin and aged extensive substrates without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v). Solid lines indicate the particle size distribution range for multi-layer extensive substrates as specified by FLL[22].

Air-filled porosity of all tested substrates complied with the FLL threshold for extensive green roofs, and differences among treatments were minor and not statistically significant (Table 2). A statistically significant decrease in hydraulic conductivity ($K_f$) was observed only at the higher SSB application rate (20% v/v), which can be attributed to the partial filling of pore spaces by fine biochar particles and increased water retention within the biochar structure itself[38]. On the contrary, $K_f$ did not decrease consistently across all biochar treatments, indicating a non-linear response to increasing SSB application rates. The pH of virgin substrates remained slightly alkaline and was not markedly affected by SSB addition, whereas

EC and TDS were significantly reduced following biochar incorporation. Particle size distributions of the virgin substrates met the FLL requirements for multi-layer extensive green roof construction (Fig. 2), with SSB-amended substrates exhibiting a slightly finer texture, particularly within the 0.1–0.5 mm fraction. Overall, SSB amendment at application rates of 10 and 20% (v/v) improved water retention-related properties, with AFP and hydraulic conductivity maintained within ranges considered acceptable for extensive green roof substrates.

Table 2. Physical and chemical characteristics of roofing substrates (mean ± SE, *n* = 3) without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v) at the beginning (10/2020) and at the end of the experiment (01/2024).

| | FLL[1] | Substrate | Age of substrate (month) | | *p*-value |
|---|---|---|---|---|---|
| | | | 0 | 40 | |
| DBD ($g\ cm^{-3}$) | | **SB0** | 1.04 ± 0.02$^{a/A}$ | 0.93 ± 0.02$^{b/X}$ | **<0.0001*** |
| | | **SB10** | 0.95 ± 0.00$^{b/B}$ | 0.86 ± 0.03$^{c/XY}$ | **<0.0001**** |
| | | **SB20** | 0.96 ± 0.01$^{b/B}$ | 0.84 ± 0.04$^{c/Y}$ | **0.0276***** |
| SBD ($g\ cm^{-3}$) | | **SB0** | 1.43 ± 0.04$^{a}$ | 1.30 ± 0.01$^{bc}$ | **<0.0001*** |
| | | **SB10** | 1.36 ± 0.01$^{ab}$ | 1.29 ± 0.01$^{bc}$ | 0.1048** |
| | | **SB20** | 1.40 ± 0.04$^{a}$ | 1.28 ± 0.03$^{c}$ | 0.4851*** |
| WHC (%) | 35 – 65 | **SB0** | 38.6 ± 1.7$^{ab/A}$ | 37.1 ± 1.1$^{a/X}$ | **<0.0001*** |
| | | **SB10** | 41.6 ± 0.1$^{bc/AB}$ | 43.4 ± 1.3$^{cd/Y}$ | **0.0137**** |
| | | **SB20** | 44.0 ± 2.0$^{cd/B}$ | 45.2 ± 0.4$^{d/Y}$ | **0.0002***** |
| $K_f$ ($mm\ min^{-1}$) | 0.6 – 70 | **SB0** | 5.0 – 8.3$^{ab/AB}$ | 29.0 – 42.3$^{c/X}$ | **<0.0001*** |
| | | **SB10** | 6.8 – 8.3$^{ab/A}$ | 13.4 – 16.1$^{b/Y}$ | **0.0343**** |
| | | **SB20** | 2.3 – 5.3$^{a/B}$ | 7.7 – 14.7$^{ab/Y}$ | **0.0010***** |
| TPo (%) | | **SB0** | 43.0 ± 2.6$^{a/A}$ | 53.9 ± 2.9$^{c}$ | **<0.0001*** |
| | | **SB10** | 46.2 ± 0.6$^{ab/A}$ | 52.9 ± 0.9$^{c}$ | **0.0038**** |
| | | **SB20** | 50.4 ± 0.7$^{bc/B}$ | 52.5 ± 0.4$^{c}$ | 0.6303*** |
| AFP (%) | □ 10 | **SB0** | 15.8 ± 4.3 | 20.2 ± 4.9 | 0.4955* |
| | | **SB10** | 20.0 ± 2.6 | 19.2 ± 2.3 | 0.3103** |
| | | **SB20** | 17.7 ± 1.6 | 17.2 ± 1.2 | 0.5404*** |
| $pH_{CaCl2}$ | 6.0 – 8.5 | **SB0** | 7.42 ± 0.03$^{a}$ | 8.12 ± 0.06$^{c/X}$ | **<0.0001*** |
| | | **SB10** | 7.55 ± 0.03$^{a}$ | 7.99 ± 0.01$^{c/Y}$ | 0.4039** |
| | | **SB20** | 7.61 ± 0.28$^{ab}$ | 7.93 ± 0.06$^{bc/Y}$ | **0.0086***** |
| EC ($mS\ cm^{-1}$) | | **SB0** | 0.72 ± 0.04$^{a/A}$ | 0.058 ± 0.001$^{c}$ | **<0.0001*** |
| | | **SB10** | 0.56 ± 0.04$^{b/B}$ | 0.058 ± 0.002$^{c}$ | **0.0006**** |
| | | **SB20** | 0.51 ± 0.01$^{b/B}$ | 0.060 ± 0.002$^{c}$ | 0.3318*** |
| TDS (ppm) | □ 3,500 | **SB0** | 360 ± 20$^{a/A}$ | 29.1 ± 0.2$^{c}$ | **<0.0001*** |
| | | **SB10** | 278 ± 22$^{b/B}$ | 28.8 ± 0.8$^{c}$ | **0.0008**** |
| | | **SB20** | 257 ± 4$^{b/B}$ | 30.2 ± 0.9$^{c}$ | 0.1089*** |
| Depth (mm) | | **SB0** | 80.0 ± 1.0$^{a}$ | 66.6 ± 1.1$^{b/X}$ | **<0.0001*** |
| | | **SB10** | 80.0 ± 1.0$^{a}$ | 72.1 ± 0.8$^{c/Y}$ | 1.0000** |

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| | | | | |
|---|---|---|---|---|
| | **SB20** | 80.0 ± 1.0$^{a}$ | 70.3 ± 0.5$^{c/Y}$ | **0.0005*** ** |

DBD – dry bulk density; SBD – saturated bulk density; WHC – water-holding capacity; $K_f$ – hydraulic conductivity; TPo – total porosity; AFP – air-filled porosity; EC – electrical conductivity; TDS – total dissolved solids.

[1]FLL specifications for a multi-layer extensive green roof media.

* for all tested substrates and age of the substrates interaction (lower case letters *a,b,c,d*); ** only for the tested substrates at the beginning (10/2020) of the experiment (capital letters *A,B*); *** only for the tested substrates at the end of the experiment (01/2024) (capital letters *X,Y*). Values shown in bold denote statistically significant treatment effects. Different lower case letters (for all tested substrates) or capital letters (individually for the substrates tested at the beginning or at the end of the experiment) at the means (mean ± SE, $n$ = 3) imply significant differences at p ≤ 0.05. Means with the same letter are not significantly different.

***Aged substrates***. A marked decline in both DBD and SBD was observed in aged substrates compared to virgin substrates, primarily attributable to the progressive weathering of substrate components, whereas differences among treatments indicate that the influence of SS biochar on bulk density was less pronounced than the effect of aging (Table 2). Conversely, the maximum water-holding capacity of aged substrates containing SSB was significantly higher than that of SB0. The WRCs for SSB-amended substrates showed a continued rightward shift (Fig. S3), extending throughout the entire pressure range up to the wilting point. Improvements in available water capacity (AWC) for plants across both biochar treatments were observed (Table S2). In the SB10, AWC increased by 40%, whereas the SB20 exhibited an increase of more than 53% (retention parameters θS, θFC, θWP, and AWC are discussed in detail in Supplementary materials 3).

Particle size distributions shifted with substrate aging, with a consistent reduction of the <2 mm fraction in aged substrates compared to virgin media (Fig. 2). This reduction was least pronounced in SB20, suggesting improved structural stability of the SSB-amended substrates. Similar age-related losses of fine particles in green roof media have been attributed to vertical migration and deposition on the geotextile layer[39]. Our rooftop experiment further shows that this process, and its hydraulic consequences, are moderated by sewage sludge biochar amendment. The simultaneous decrease in dry bulk density (DBD) and substrate depth after 40 months is consistent with the reduction of the <2 mm fraction, indicating progressive structural reorganization of the substrate profile. The more pronounced depth loss in SB0 compared to SSB-amended treatments (Table 2) suggests that the incorporation of SSB moderated the structural changes under rooftop conditions. Moreover, the changes in grain size are known to be reflected in changes in $K_f$[40]. Overall, the aged substrates had higher values of $K_f$ than their virgin counterparts. Although aging increased $K_f$ across treatments, SSB-amended substrates maintained substantially lower $K_f$ than aged SB0 (Table 2), indicating that biochar addition moderated the age-driven increase in hydraulic conductivity. Our results indicate that substrate aging is the dominant factor in structural and hydraulic changes in extensive green roof substrates. SSB amendment modified this aging process by reducing fine particle loss, limiting substrate thinning, and mitigating increases in hydraulic conductivity associated with substrate aging.

Air-filled porosity of all tested substrates complied with the FLL guidelines for extensive green roof media (Table 2). Substrate aging was associated with an increase in AFP in SB0, whereas biochar-amended substrates exhibited a more stable pore characteristics over time. While differences in TPo among the aged substrates were not statistically significant, all tested substrates exhibited higher TPo levels after 40 months compared to the virgin substrates, with the most pronounced increase occurring in the substrate without biochar. Similar long-term increases in porosity of extensive green roof substrates have been attributed to combined physical (water flux, drying–wetting cycles) and biological processes (root development, microbial activity)[41]. From a chemical perspective, substrate pH showed a modest increase with aging across all treatments, with aged biochar-amended substrates (SB10 and SB20) maintaining significantly lower pH values than aged SB0. However, in the long term, substrates for green roofs appear to maintain a preferred pH range of 6.0-8.5[22]. EC and TDS values substantially decreased over time when comparing virgin and aged substrates, irrespective of the incorporation of SSB.

## Experimental green roof monitoring

### *Runoff quantity and substrate moisture*

The studied years (2020–2023) differed markedly in their climatic conditions. From October 2020 to December 2023, 250 daily rainfall depths were recorded, of which 210 were categorized as low (1 – 9.9 mm), 37 as moderate (10 – 24.9 mm), and 8 as high daily rainfall depths ( 25 mm). Total precipitation amounted to 1845.7 mm, with 2023 being the rainiest year (667.5 mm) and 2022 the driest (479.5 mm).

As shown in Fig. 3, runoff from the experimental green roof plots varied substantially among years, primarily reflecting differences in annual rainfall amount, intensity, and distribution, as well as the progressive development of vegetation cover. Runoff was generated predominantly during days with moderate and high daily rainfall totals. The very rainy period at the start of the experiment (11–15 October 2020; 91.4 mm) resulted in pronounced differences in runoff among treatments, which was reflected in significantly different runoff reduction values (Table 3). Although partial precipitation compensation was applied, the initial imbalance likely affected nutrient leaching in the early stages (see Fig. 5F). However, treatment differences in hydrological performance and structural parameters persisted over subsequent years, suggesting that long-term trends were not governed by the installation variability.

Over the entire study period, SSB amendment at both application rates was associated with reduced runoff and improved retention capacity, with mean reductions of 3.3–5.6% for SB10 and 7.4–8.5% for SB20 compared to the control. Annual runoff reduction ranged from 43.1–68.8% (SB0), 48.7–72.1%

(SB10), and 50.7–76.2% (SB20), with 2022, the year with the lowest cumulative annual precipitation, showing the highest overall water retention. The cumulative divergence in runoff volumes among treatments over the entire monitoring period is illustrated in Supplementary Fig. S4. Seasonal runoff reduction was highly variable and strongly dependent on rainfall, with the highest absolute reductions typically observed during summer, particularly in SSB-amended substrates. These observations are consistent with the findings of Tan and Wang[24], who reported approximately 20% greater runoff reduction in green roofs amended with 10–20% (w/w) wood-based biochar compared to unamended systems.

The addition of SSB affected the moisture content of substrates, although there were considerable intra- and inter-annual variations. Immediately after installation (10/2020), biochar-amended substrates (SB10 and SB20) exhibited higher moisture contents than SB0, consistent with the increased WHC of fresh SSB substrates. The more pronounced moisture differences observed in 2020 reflect the establishment phase and runoff responses specific to treatments following installation. These differences gradually diminished as substrate physical properties stabilized and vegetation cover developed over time. In autumn and winter months, moisture fluctuations were lower, reflecting wetter conditions and reduced evaporative demand. During spring and summer, substrate moisture decreased rapidly after precipitation events, with consistently lower values in SB0 compared to SB10 and SB20 (Fig. 3). In the first study year, after the rain events, substrate SB20 reached markedly higher absolute moisture levels, which coincided with cooler temperatures and lower atmospheric evaporative demand during the establishment phase. These climatic conditions likely contributed to moisture persistence in addition to the higher WHC of SSB-amended substrates. However, as the *Sedum* cover increased over time, the differences in moisture content between the SB20 and SB10 substrates gradually diminished. In the summers of 2022 and 2023, we observed higher absolute substrate moisture contents for SB10, which is related to greater *Sedum* cover compared to the other substrates (Fig. 6) because better plant cover leads to less evaporative losses from the substrate surface. This is consistent with Nektarios et al.[42] and Getter and Rowe[43], who found that the absolute cover of *Sedum* plants was higher on substrates with higher volumetric moisture. Jelínková et al.[44] emphasized that the increase in water retention (and consequently the moisture content) results from the interacting effects of *Sedum* plant cover, substrate properties, and weather conditions.

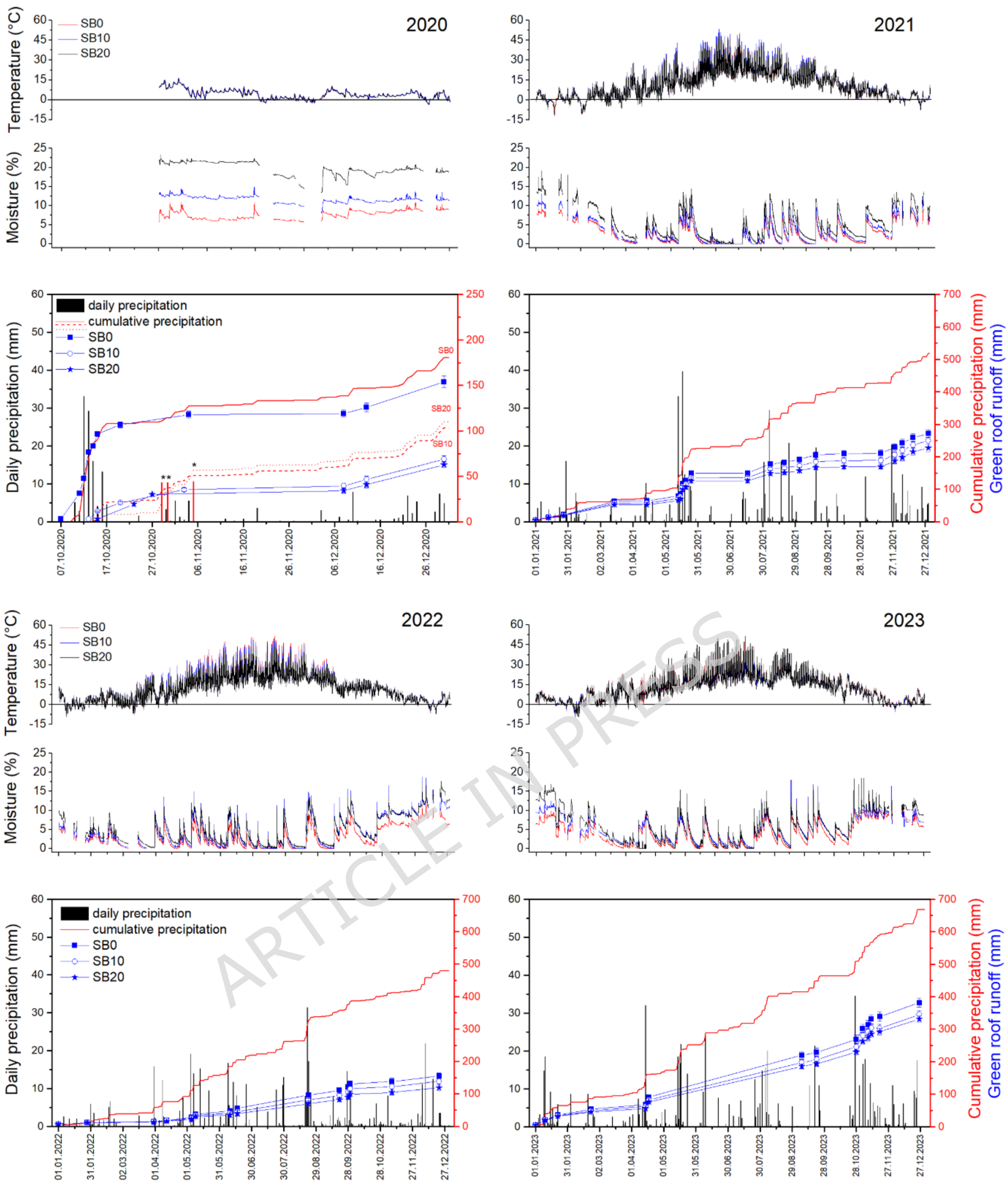


Fig. 3. Temperature and moisture of extensive substrates without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v) measured 3 cm below the surface during the four years of experiment (10/20 – 12/23). Cumulative precipitation, daily precipitation, and mean runoff from experimental green units are shown below the temperature and moisture plots. Because moisture sensors cannot function at temperatures below 0°C, these data points were excluded. * indicate the addition of rainwater (red columns) to SB10 (10 mm) and SB20 (30 mm) to balance the differences in precipitation at the experiment set-up. These additions were accounted for in the precipitation sums used for runoff reduction calculations.

Direct comparison of biochar effect on absolute substrate moisture values after rainfall across studies is challenging, primarily due to differences in substrate depth and sensor placement. In the present study, maximum substrate moisture after rainfall in SB10 and SB20 ranged from 10 to 22% (substrate depth 80 mm, sensor placement at 30 mm below the surface), which is comparable to values reported by Kuoppamäki et al.[13], who observed maximum moisture contents of biochar substrates ranging from 10 to 20% (substrate depth 40 mm + 30 mm *Sedum* mats, sensor placement 20 mm). Substantially higher moisture (40–45%) was reported for ROOFChar® substrates amended with 5% (v/v) wood-based biochar, measured at greater (50 mm) substrate depth[20], highlighting the strong influence of measurement configuration on absolute moisture values.

Table 3. The annual and seasonal amount of runoff reduction (mm) and runoff reduction (%) of experimental green roofs without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v) during the period of 2020 - 2023.

| **Year** | | **Annual precipitation (mm)** | **Runoff reduction (mm)** | | | **Runoff reduction (%)** | | |
|---|---|---|---|---|---|---|---|---|
| | | | SB0 | SB10 | SB20 | SB0 | SB10 | SB20 |
| 2020 | | 180.6* | 27.7 | 36.5** | 49.2** | 15.3 | 35.3 | 44.8 |
| 2021 | | 518.2 | 250.0 | 272.4 | 294.0 | 48.2 | 52.6 | 56.7 |
| 2022 | | 479.5 | 329.8 | 345.7 | 365.6 | 68.8 | 72.1 | 76.2 |
| 2023 | | 667.5 | 287.6 | 324.9 | 338.4 | 43.1 | 48.7 | 50.7 |
| **Year/season** | | **Seasonal precipitation (mm)** | **Runoff reduction (mm)** | | | **Runoff reduction (%)** | | |
| | | | SB0 | SB10 | SB20 | SB0 | SB10 | SB20 |
| 2020 | Autumn | 133.4 | 16.0 | 19.4** | 31.0** | 12.0 | 34.5 | 49.6 |
| | Winter | 47.2 | 11.7 | 17.1 | 18.2 | 24.8 | 36.2 | 38.5 |
| 2021 | Spring | 164.9 | 36.8 | 49.1 | 57.7 | 22.3 | 29.8 | 35.0 |
| | Summer | 141.2 | 107.3 | 110.9 | 115.9 | 76.0 | 78.5 | 82.1 |
| | Autumn | 93.0 | 45.9 | 51.6 | 56.4 | 49.4 | 55.5 | 60.7 |
| | Winter | 119.2 | 60.0 | 60.8 | 64.0 | 50.3 | 51.0 | 53.7 |
| 2022 | Spring | 118.1 | 91.5 | 95.5 | 98.8 | 77.5 | 80.9 | 83.7 |
| | Summer | 180.9 | 121.4 | 132.4 | 140.9 | 67.2 | 73.2 | 77.9 |
| | Autumn | 81.0 | 38.7 | 40.0 | 46.5 | 47.8 | 49.4 | 57.4 |
| | Winter | 99.6 | 78.2 | 77.8 | 79.4 | 78.5 | 78.1 | 79.7 |
| 2023 | Spring/ Summer*** | 195.3/ 128.3 | 156.0 | 173.5 | 182.5 | 48.2 | 53.6 | 56.4 |
| | Autumn | 180.8 | 60.8 | 74.4 | 73.2 | 33.6 | 41.2 | 40.5 |
| | Winter | 163.0 | 70.8 | 77.0 | 82.7 | 43.5 | 47.2 | 50.7 |

* cumulative precipitation from the experiment establishment in 10/2020; ** cumulative precipitation of 103.5 mm (SB10) and 109.7 mm (SB20) and autumn precipitation of 56.2 mm (SB10, autumn) and 62.5 mm (SB20, autumn) were applied due to the delayed installation of experimental plots because of unfavorable weather conditions; *** during summer 2023, due to technical constraints, runoff volumes were measured cumulatively over the April–August period rather than after individual events.

Our long-term study confirms that SSB amendment increases WHC of green roof substrates and is associated with higher substrate moisture under rooftop conditions, with effects comparable to those reported for plant-based biochars. Previous studies observed increases in WHC of 4 and 10% following agricultural[11] and wood-based[13] biochar application, with improvements in runoff retention performance observed across a range of rainfall conditions. Under ongoing climate change, manifested in the southern part of Slovakia by a 10% decrease in annual precipitation and increasing rainfall variability and extremes[45,46], significant differences in substrate moisture and runoff retention are expected to vary not only interannually but also seasonally (e.g., in spring 2021 and spring 2022, Fig. 3, Table 3). These dynamics underline the importance of considering both substrate composition and hydrological variability in green roof substrate design.

### *Water balance*

Daily changes in water storage within the lysimetric green roof plots differed among treatments and seasons, reflecting variations in atmospheric evaporative demand and substrate water retention. Fig. 4 compares these changes with the daily sums of potential evapotranspiration ($ET_0$) estimated by the Penman-Monteith equation (1) during autumn 2021 and spring and summer 2022. $ET_0$ was calculated as a reference indicator of atmospheric evaporative demand to support the interpretation of measured evapotranspiration $ET_{lys}$ and to distinguish between energy- and water-limited conditions. Positive values indicate increasing water storage following rainfall events. Higher water storage levels in SB10 and SB20 are generally consistent with the increased WHC associated with SSB amendment and suggest improved retention under low- to moderate-intensity rainfall.



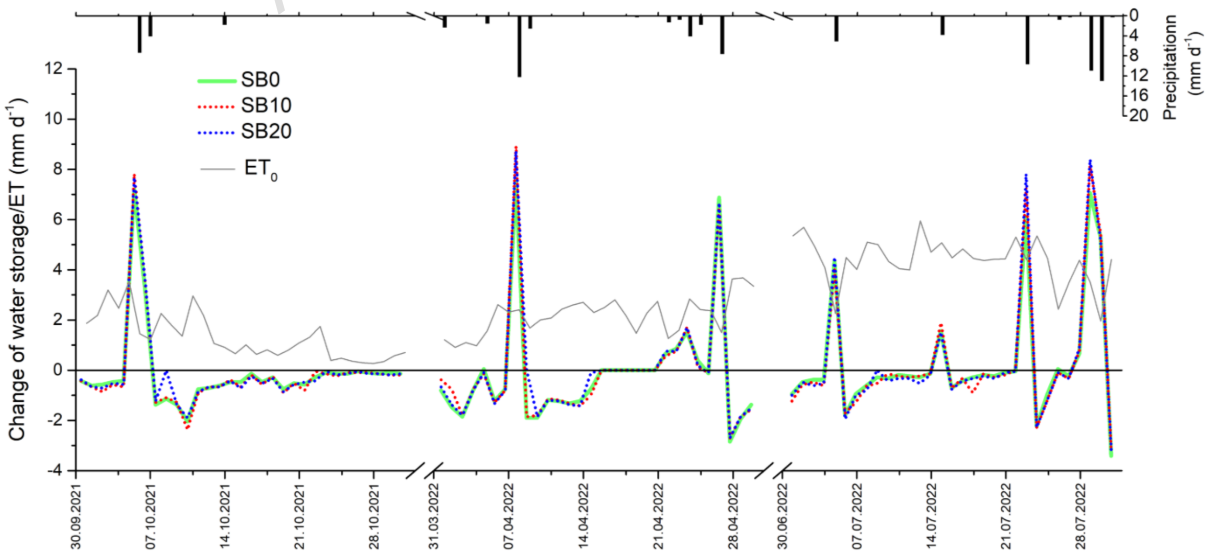


Fig. 4. Daily precipitation, potential evapotranspiration ($ET_0$) estimated using the Penman-Monteith equation, and change of water storage (mm $d^{-1}$) in the

experimental green roofs without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v) during autumn 2021, spring and summer 2022. Weight change data were unavailable from 15 to 20 April 2022 due to a temporary lysimeter error.

Measured $ET_{lys}$ values (expressed as negative changes in water storage, Fig. 4) were consistently lower than $ET_0$ during autumn (mean 1.3 mm $d^{-1}$) and spring (mean 2.2 mm $d^{-1}$), indicating an insufficient amount of water available for evapotranspiration. Mean $ET_{lys}$ values did not differ markedly among substrates. However, the effect of biochar on the short-term changes in water storage can be observed, especially following days with large daily rainfall depths, and is consistent with lower runoff and higher moisture in SB10 and SB20 substrates (Fig. 3). During summer, $ET_0$ reached its highest values (mean 4.4 mm $d^{-1}$). However, $ET_{lys}$ remained low, likely reflecting limited water availability in the substrate and reduced evapotranspiration under limited water conditions that may lead to *Sedum* plant stress. Practically immediately after the precipitation, substrate moisture started to decline via evapotranspiration. It was almost entirely depleted after a few days regardless of the biochar amendment (Fig. 4). Berghage et al. [47] confirmed that *Sedum* plants have the ability to improve evapotranspiration rates from green roofs, especially when water is readily available (e.g. after rain events). These plants can store approximately 1 to 10 mm of water within their leaves, which can be utilized during periods of drought[48].

While SSB addition increased WHC and substrate moisture, the absolute daily retention volumes remained relatively low, with maximum retention of approximately 8 mm. This indicates that SSB-amended substrates can extend periods of favorable moisture availability that reduce plant stress (during periods with small to medium daily rainfall depths), but their capacity to fully retain runoff during high-intensity precipitation is limited. Such retention behavior is consistent with the function and design of shallow extensive green roofs, which are not intended to fully mitigate extreme storm events[49]. Nevertheless, improved substrate storage capacity and cumulative reductions in runoff volumes over time represent a meaningful contribution to overall hydrological performance, particularly by reducing runoff frequency and volume during more common moderate rainfall events in all seasons. The broader environmental benefits of SSB amendment are further supported by the preliminary life cycle assessment presented in Supplementary materials S4.

### *Runoff quality*

The addition of SSB tended to maintain or slightly elevate the pH of runoff relative to SB0, with no substantial differences in runoff pH detected across years (Fig. 5A). The range between minimum and maximum pH values within individual years reflects both inter- and intra-annual variability, likely associated with progressive weathering of substrate components and seasonal effects such as freezing and thawing cycles (Fig. 3)[16].

Values of EC recorded following the first leaching events in 2020 were significantly higher than those observed in subsequent periods (2021–2023), indicating an initial first-flush response during early substrate wetting, consistent with previous observations[50]. The EC declined below 1 mS $cm^{-1}$ in 2021 and subsequently fluctuated between 0.08 and 0.38 mS $cm^{-1}$ during 2022 and 2023. Although SSB amendment initially resulted in higher EC and COD values in runoff compared to SB0, especially during the establishment phase, these differences diminished over time and remained minor among treatments (Fig. 5B, C). TSS concentrations were relatively low across all treatments, with median values consistently below 2 mg $L^{-1}$ during the first year (Fig. 5D), indicating no significant effect of SSB on particulate retention. In 2021 and 2022, TSS values remained relatively stable however a higher frequency of outliers was observed. These episodic fluctuations may be associated with seasonal vegetation developments. A pronounced increase in TSS concentrations was recorded in 2023 across all treatments. This increase followed the winter period and may reflect physical deterioration of substrate aggregates due to repeated freeze–thaw cycles, leading to temporary mobilization of fine particles during subsequent rainfall events[51].

Nutrient concentrations in the runoff exhibited a declining trend over the 40-month monitoring period. Higher TN and TP concentrations compared to SB0 in 2020 (TN and TP) and, in the case of TP, also in 2021 were observed in SSB-amended substrates (Fig. 5E,F). Nitrogen was leached more rapidly than phosphorus, whereas phosphorus concentrations decreased more gradually over time, consistent with the generally lower mobility of phosphorus compared to nitrogen in such substrates[52]. Compared to SB0, significantly elevated TN concentrations were observed in 2020, with median values exceeding 30 mg $L^{-1}$ for SB10 and SB20. These values indicate elevated nitrogen export during the establishment phase. However, the substantially higher TN concentrations observed in SB10 and SB20 in 2020 should be interpreted in the context of differences in runoff volume among treatments during this period. As shown in Fig. 3 and Table 3, cumulative precipitation and corresponding runoff volumes differed markedly in 2020, with SB0 receiving substantially higher rainfall (180.6 mm) than SB10 and SB20 (103.5 and 109.7 mm). Although partial rainfall equalization was applied during installation, runoff from SB10 and SB20 remained markedly lower than from SB0. Lower runoff volumes likely contributed to higher measured TN concentrations (mg $L^{-1}$) in SSB-amended treatments during this period. From 2021 onward, TN concentrations decreased markedly across all treatments, with no significant distinctions among substrates by 2022 and 2023 (Fig. 5F). This rapid decline and stabilization suggest the exhaustion of easily leachable nitrogen fractions, along with the establishment of vegetation (Fig. 6) and microbial communities capable of nitrogen uptake or transformation. The elevated TN concentrations were limited to the establishment phase and did not represent sustained long-term leaching or progressive deterioration of runoff quality under the monitored rooftop conditions.

Moreover, the initial increase in TP observed in biochar-amended plots is in line with reports of initial release of labile phosphorus fractions from freshly applied biochar materials[51]. The differences in TP concentrations among the treatments decreased over time, and by 2023, median TP values converged across all substrate types, irrespective of biochar content. This pattern suggests temporal stabilization rather than progressive increases in phosphorus leaching. Similar initial nutrient dynamics have been reported in green roof systems during the first year following installation[6].

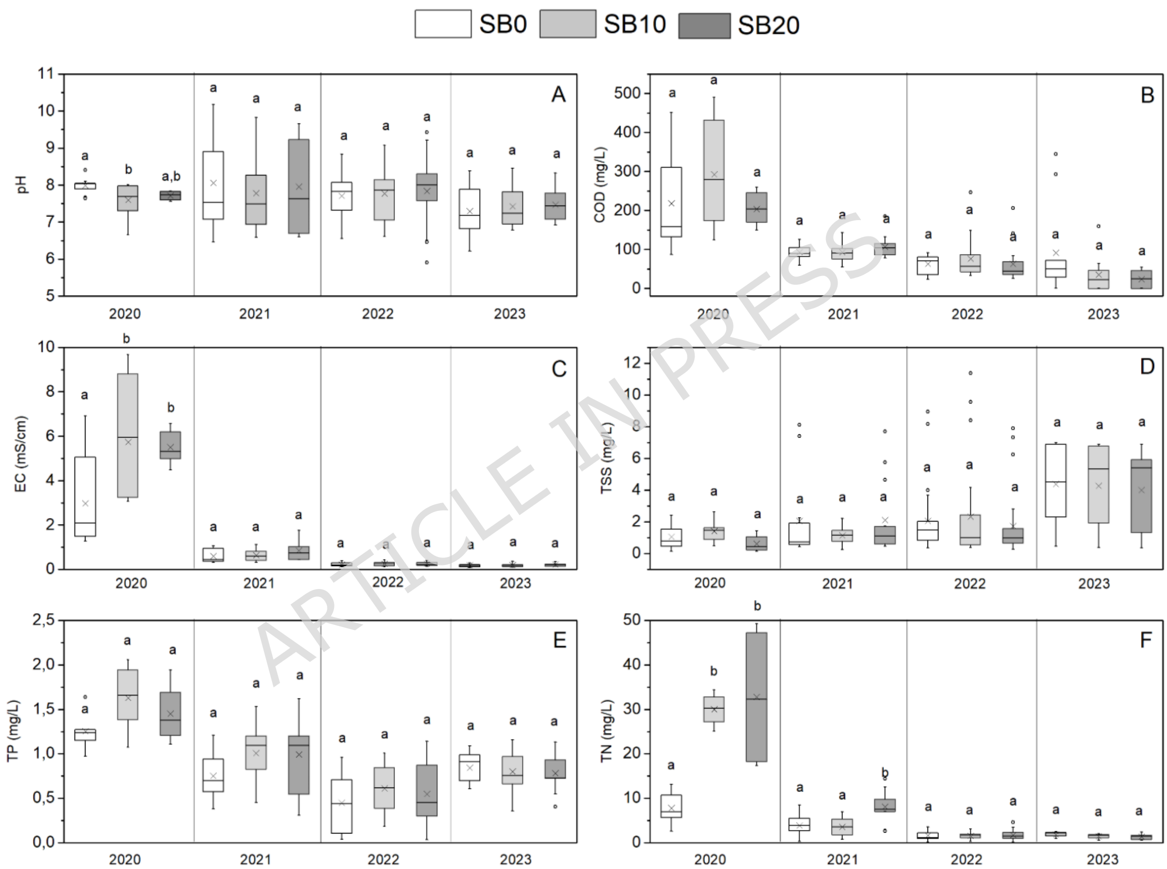

Fig. 5. Box-whisker plots of the pH, COD, EC, TSS, and nutrients of runoff from experimental green roof substrates without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v) during four years. Box = interquartile range (IQR); horizontal line = median; × = mean; whiskers = 1.5 × IQR; points beyond whiskers = outliers. Sample sizes (n) for panels A, C, D: 2020 – SB0 (13), SB10 (11), SB20 (8); 2021 – all treatments (16); 2022 – all treatments (26); 2023 – all treatments (16). Sample sizes (n) for panels B, E, F: 2020 – SB0 (11), SB10 (8), SB20 (7); 2021 – all treatments (14); 2022 – all treatments (16); 2023 – all treatments (14). Different lower case letters imply significant differences between treatments within a given year according to Tukey's Honestly Significant Difference (HSD) post hoc test at $p \leq 0.05$. Means with the same letter are not significantly different.

Previous research has demonstrated that plant-based biochar is frequently utilized as a substrate additive to mitigate runoff volume[12,14], and field-scale studies have demonstrated reductions in phosphorus and nitrogen leaching following biochar application[53]. Liao et al.[54] reported that runoff from pre-grown sedum mat green roofs amended with maple sugar-based biochar (5% v/v) exhibited improved overall water quality, which was attributed to the combined effects of biochar's strong nutrient and ion sorption capacity and dense vegetation cover. Conversely, biochar derived from nutrient-rich feedstocks, such as sewage sludge, may exhibit early-stage nutrient release during leaching following rainfall events[5], as reflected in the initial monitoring period of the present study.

**Vegetation cover**

The temporal development of the *Sedum* vegetation differed among treatments over the monitoring period (Fig. 6). Initially, the state of vegetation was uniform across all plots following installation. However, differences in the coverage and abundance of *Sedum* species gradually emerged over the monitoring period. After 24 months (September 2022), SB10 exhibited the highest cover, and by October 2023 total vegetation cover reached 73% in SB0, compared to 89% and 84% in SB10 and SB20, respectively. Although both SSB-amended substrates showed significantly higher cover than SB0, the difference between SB10 and SB20 was not statistically significant, indicating a non-linear response to SSB rate (Fig. 6). Across the study period, the species composition shifted toward the dominance of *S. sexangulare* and *S. spurium*, while *S. spurium 'Voodoo'* declined, with no clear differences in species dominance observed between treatments.

Higher vegetation cover in SSB-amended substrates was observed along with higher substrate moisture content and improved water retention properties described in the previous sections, indicating a potentially less water-limited growth environment under real rooftop conditions. However, the response of *Sedum* vegetation was not strictly dose-dependent, as the highest mean cover was observed at 10% (v/v) SSB, whereas 20% (v/v) did not result in a further increase in vegetation cover. Previous studies have shown that SSB amendment can influence plant performance primarily through combined effects on substrate moisture regime and substrate chemical properties (e.g. pH and nutrient composition and availability)[5,15]. Vegetation dynamics were also consistent with substrate ageing patterns described (see the section Aged substrates), where greater substrate thinning and structural change were observed in SB0 compared to SSB-amended substrates. Long-term studies have demonstrated that substrate depth and structural stability play an important role in maintaining vegetation cover on extensive green roofs, particularly in environments prone to drought[41].

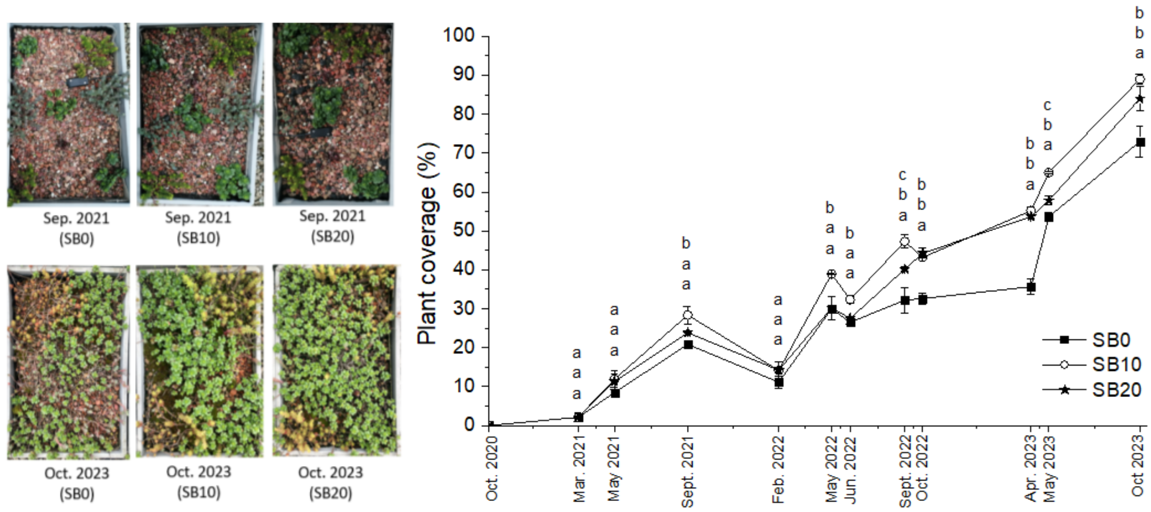


Fig. 6. Development of *Sedum* plant vegetation (*S. reflexum, S. sexangulare, S. spurium, S. spurium voodoo*) over time in experimental green plots with extensive substrates without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v). Different lower case letters imply significant differences between treatments according to Tukey's Honestly Significant Difference (HSD) post hoc test at $p \leq 0.05$. Means with the same letter are not significantly different.

However, the observed vegetation patterns likely reflect the combined influence of multiple interacting factors rather than a single controlling factor, including interannual weather variability and substrate physical evolution. In addition, the non-linear response to SSB incorporation suggests that moderate amendment rates may provide a more favorable balance between moisture retention and substrate aeration, whereas higher SSB proportions do not necessarily result in further increases in vegetation cover. This interpretation is consistent with long-term green roof studies showing that *Sedum* cover dynamics are strongly mediated by water availability and substrate configuration under real rooftop conditions[43].

## Microbial diversity in green roof substrates

Alpha diversity indices revealed differing patterns of bacterial and fungal communities to SSB amendments in green roof substrates. For bacteria (Fig. 7A–C), the SB10 treatment showed high variability across replicates, while SB0 and SB20 displayed more consistent values. A significant decrease in diversity index was recorded only in 2022, where SB20 showed reduced community evenness ($p \leq 0.05$), suggesting dominance by fewer bacterial taxa under prolonged high biochar exposure. Interestingly, despite the lack of statistical significance, the high biochar amendment (SB20) generally resulted in increased values of diversity indices such as Simpson and Shannon for both bacterial and fungal communities. On the contrary, the 10% biochar treatment (SB10) was associated with greater intra-treatment variability and lower mean diversity values compared to the control (SB0). These observations diverge from previous findings by Chen et al.[3,15], who reported significant increases in both microbial abundance and alpha diversity indices following the application of SSB in soil-based systems in green roof ecosystem. Fungal alpha diversity (Fig. 7D–F) was more uniform in 2021 across treatments, whereas variability within treatments increased in 2022, especially in SB0 and SB10. A significant difference was found in the Simpson index in 2021, where SB0 exhibited lower fungal diversity than biochar treatments, suggesting early stabilizing effects of SSB on fungal communities.

A plausible explanation for the reduced evenness under SB20 in 2022 is that higher biochar dosage may selectively favour microbial groups adapted to biochar-enriched microhabitats[55]. Increased porosity, higher specific surface area, altered pH buffering capacity, and additional labile carbon fractions from SSB can create relatively stable niches that promote the proliferation of certain fast-growing or biochar-adapted taxa, potentially leading to competitive dominance and reduced evenness[55,56]. In contrast, the intermediate SB10 treatment may have generated a more spatially heterogeneous substrate matrix, where biochar particles were unevenly distributed. This could be a result of patch-scale variability in moisture retention, nutrient availability, and microsite conditions. Also, such heterogeneity could support different microbial assemblages across replicates, thereby explaining the higher intra-treatment variability observed for SB10.

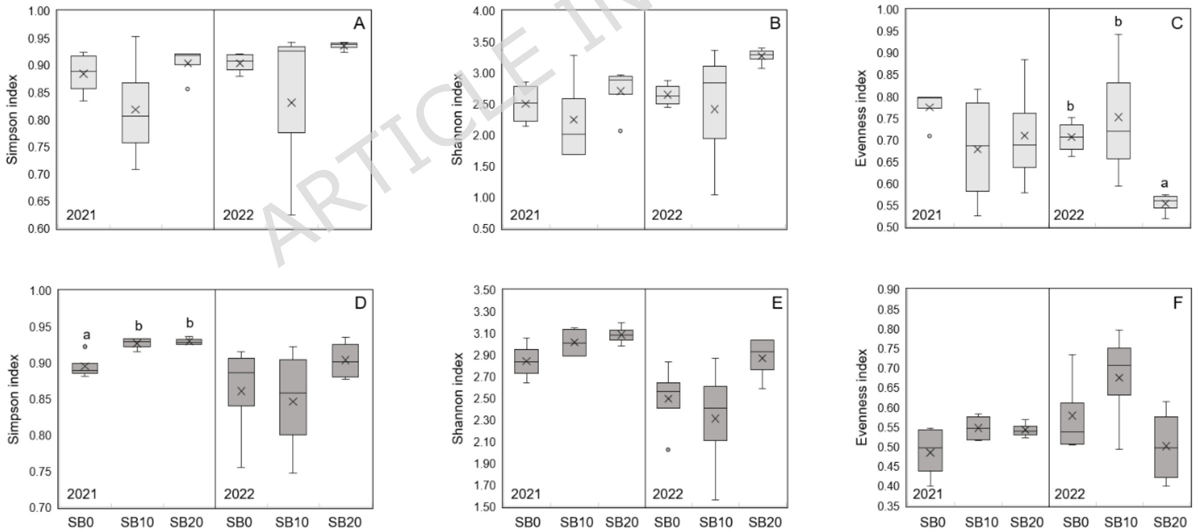




Fig. 7. Alpha diversity indices of bacterial (A–C) and fungal (D–F) communities in green roof substrates without (SB0) and with the addition of 10% (SB10) and 20% (SB20) sewage sludge biochar (v/v) in two consecutive years (2021 and 2022). Simpson (A, D), Shannon (B, E), and Evenness (C, F) indices were calculated from ARISA fragment profiles. Distinct lowercase letters denote statistically significant differences between treatments within a given year according to LSD *post hoc* test ($p \leq 0.05$).

The UpSet plots (Fig. 8) illustrate the distribution of observed operational taxonomic units (OTUs) across all treatment combinations (SB0, SB10, SB20) and sampling years (2021 and 2022), highlighting both shared and unique components of microbial richness. For bacterial communities, the SB20_2022 group contained the highest number of unique OTUs (n = 42), followed by SB20_2021 (n = 41), indicating that the highest biochar dose consistently supported a broader taxonomic repertoire across both years. Conversely, lower richness and fewer unique OTUs were observed in SB10 treatments, particularly in SB10_2021 and SB10_2022, supporting previous observations of reduced alpha diversity under intermediate biochar application. For fungal communities, OTU richness was more evenly distributed across treatments in 2021, with no single treatment showing dominant exclusivity. However, in 2022, a marked decline in unique OTUs was detected in SB10_2022, which had one of the lowest intersection sizes, whereas SB20_2022 maintained a relatively high number of unique taxa (n = 30), suggesting improved taxonomic stability under high biochar amendment.

These findings reinforce the patterns observed in the alpha diversity indices and highlight the dose-dependent and temporal impacts of biochar on microbial richness in green roof systems. Our results correspond with meta-analyses by Singh et al.[57], which showed that biochar can improve microbial diversity but with differential responses between bacteria and fungi. They noted that fungal diversity often increases more slowly than bacterial diversity and may be more strongly influenced by longer-term variations in soil physical and chemical properties, such as improved porosity and pH stabilisation. This supports the temporal dynamics observed in our experiment, where fungal community enhancement in SB20 became apparent only after two years. The increased diversity of bacterial communities, as measured by the OTUs richness and Chao1 index, following the application of biochar can be attributed to the carbon sources and ash inputs found in the nutrient-rich SSB. These new carbon sources may serve as substrates for various bacterial phyla or species [58,59].

Beyond nutrient supply, structural, physical, and chemical mechanisms likely contributed to the observed microbial responses. The increase in microbial richness and biomass observed in SSB-amended substrates can be interpreted in light of structural, physical, and chemical mechanisms described for biochar-amended soils. Biochar addition has been shown to modify pore size distribution and increase total porosity, particularly within the >5 μm pore fraction, which positively influences microbial diversity and abundance by improving habitat heterogeneity and aeration[60]. These structural changes enhance the availability of protected microsites that support microbial colonization. Changes in pore architecture and connectivity have also been linked to shifts in microbial community structure and carbon transformation processes. Zhang et al.[61] demonstrated that biochar-induced alterations in pore structure were closely associated with changes in microbial diversity and carbon mineralization patterns, indicating that physical restructuring of the substrate can regulate microbial ecological dynamics. Similarly, Zhang et al.[62] demonstrated in a meta-analysis of 49 studies that biochar structural properties, particularly specific surface area and porosity, are key determinants of increased total microbial biomass and the activity of bacteria, fungi, and actinomycetes. They concluded that higher biochar application rates provide greater surface area and pore space, enhancing water and nutrient retention and thereby promoting microbial abundance and activity.

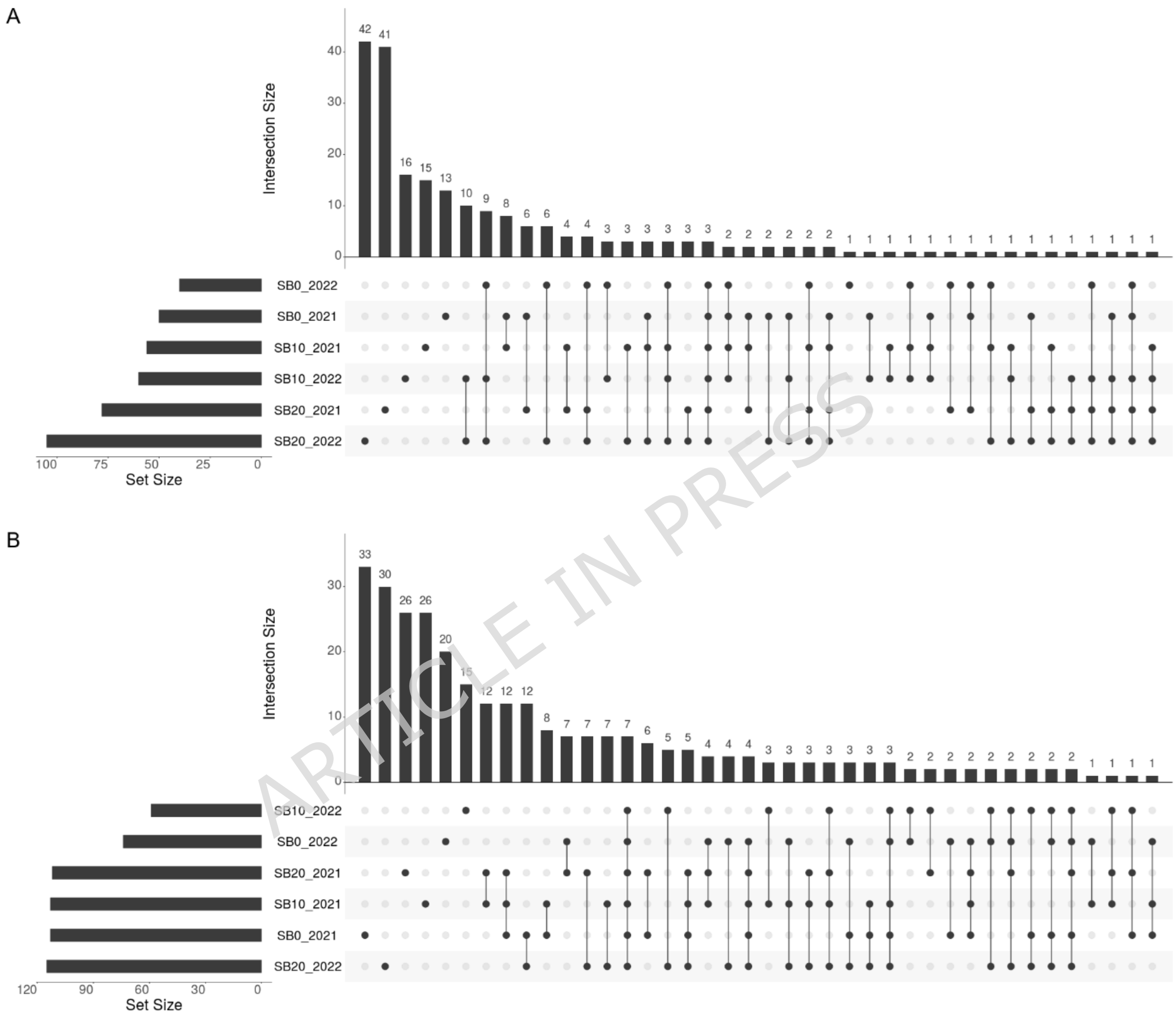


Fig. 8. UpSet plots showing the richness and overlap of operational taxonomic units (OTUs) among treatments and sampling years in green roof substrates; (A) Bacterial richness based on B-ARISA profiles; (B) Fungal richness based on F-ARISA profiles. Each horizontal bar on the left represents the total number of OTUs detected in a given treatment-year combination. Vertical bars indicate the number of shared or unique OTUs in specific set intersections, as denoted by the connected dots below.

Overall, the observed increase in microbial richness is consistent with prior evidence that biochar improves soil habitat heterogeneity and nutrient availability, thereby supporting more functionally diverse microbial assemblages[63]. However, the decline in fungal richness in SB10 in the second year suggests that moderate amendment rates may not sustain long-term benefits for all microbial groups, possibly due to competition, microbial succession, or physical and chemical shifts unfavourable to fungi. Based on a meta-analysis of 999 paired data points across 194 studies, Li et al.[63] demonstrated that biochar generally promotes microbial biomass, although its impacts on microbial diversity can vary. They found that the effect of biochar on microbial biomass is determined mainly by the specific properties of the biochar, while soil characteristics predominantly affect microbial diversity. In our experiment, UpSet plots revealed a higher richness partitioning among fungal communities and a greater overlap among bacterial communities, consistent with previous findings that bacterial taxa tend to colonise substrates more broadly, while fungal colonisation is more selective[57,61].

**Structural equation modelling and path analysis**

Multiple structural equation models (SEMs) were specified a priori to evaluate potential associations and statistically mediated relationships linking SSB dose, year, runoff characteristics, and microbial richness. Model construction followed a hierarchical approach beginning with a full model including all measured runoff parameters (pH, EC, TN, TP, COD, TSS) and plant cover. Due to limited sample size ($N = 24$) and the high number of free parameters, the full model showed poor global fit and evidence of overparameterization (see Supplementary Table S5). Subsequent model reduction was performed by removing variables contributing to model instability and poor fit, guided by theoretical plausibility and statistical criteria (RMSEA, CFI, SRMR, AIC, BIC). The final parsimonious model retained pH, EC, and TSS (Fig. 9) as mediating variables and demonstrated good global fit ($\chi^2 = 3.33$, $p = 0.344$; RMSEA = 0.067; CFI = 0.998; SRMR = 0.012). The simplified path model explained 62% of the variance in bacterial richness ($R^2 = 0.620$) and 70% in fungal richness ($R^2 = 0.695$).



Because pH, EC, and TSS were measured in the runoff water and not within the substrate matrix itself, they are interpreted as integrative indicators of leaching dynamics and system-level physical and chemical gradients. In the SEM framework, these variables represent treatment- and time-associated environmental signals within the green roof system, and therefore, they are not interpreted as direct drivers of microbial richness. A comparable explained variance in bacterial richness (62%) due to SSB amendment was also reported in the path analysis by Chen et al.[3]. In their study, fungal communities exhibited even higher explained variability, reaching up to 85%. However, their findings indicated a negative standardized coefficient for the effect of biochar on bacteria, suggesting a suppressive influence. Our application of 20% biochar showed a strong positive standardized path coefficient with bacterial richness ($\beta = 0.805$, $p < 0.001$) and a moderate positive association with fungal richness ($\beta = 0.389$, $p = 0.022$), corroborating observations from the UpSet plot analysis. In contrast, EC in the runoff had a significant negative association with bacterial richness ($\beta = -0.794$, $p = 0.027$), suggesting that increased leaching of dissolved ions may reflect stress conditions that suppress bacterial abundance. Similarly, TSS negatively impacted fungal richness ($\beta = -0.392$, $p = 0.016$), indicating that greater particulate matter loss may impair fungal colonization or stability. Furthermore, interannual variation (2022 vs. 2021) significantly reduced fungal richness ($\beta = -0.730$, $p = 0.007$) and indirectly influenced bacterial richness via EC (indirect $\beta = 0.708$, $p = 0.029$) and fungal richness via TSS (indirect $\beta = 0.257$, $p = 0.032$). These indirect associations suggest that interannual variation and changes in runoff characteristics related to treatment occur together with shifts in microbial richness patterns within the monitored system.

Our findings are consistent with broader research. For instance, Chen et al.[15] also observed that the path coefficient of biochar on soil properties reached 0.89, whereas its effect on soil microorganisms was substantially lower (0.22). In their experiment, this suggested that biochar exerted a more pronounced direct impact on soil physical and chemical characteristics (soil porosity, available N, P, K, total carbon, etc.) than on the soil microbial communities. Zhang and Shen[64] reported that the structure of microbial communities in soils amended with varying amounts of biochar was primarily determined by moisture content, porosity, potassium fertilizer, and nitrogen fertilizer, rather than by organic matter and phosphate fertilizer. Other studies have similarly shown that biochar amendment can influence microbial community composition[60, 65,66]. For instance, Yang et al.[60] demonstrated that biochar amendments affected both the diversity of bacterial and fungal communities in soils, and this effect depended on the biochar's feedstock type and pyrolysis temperature.

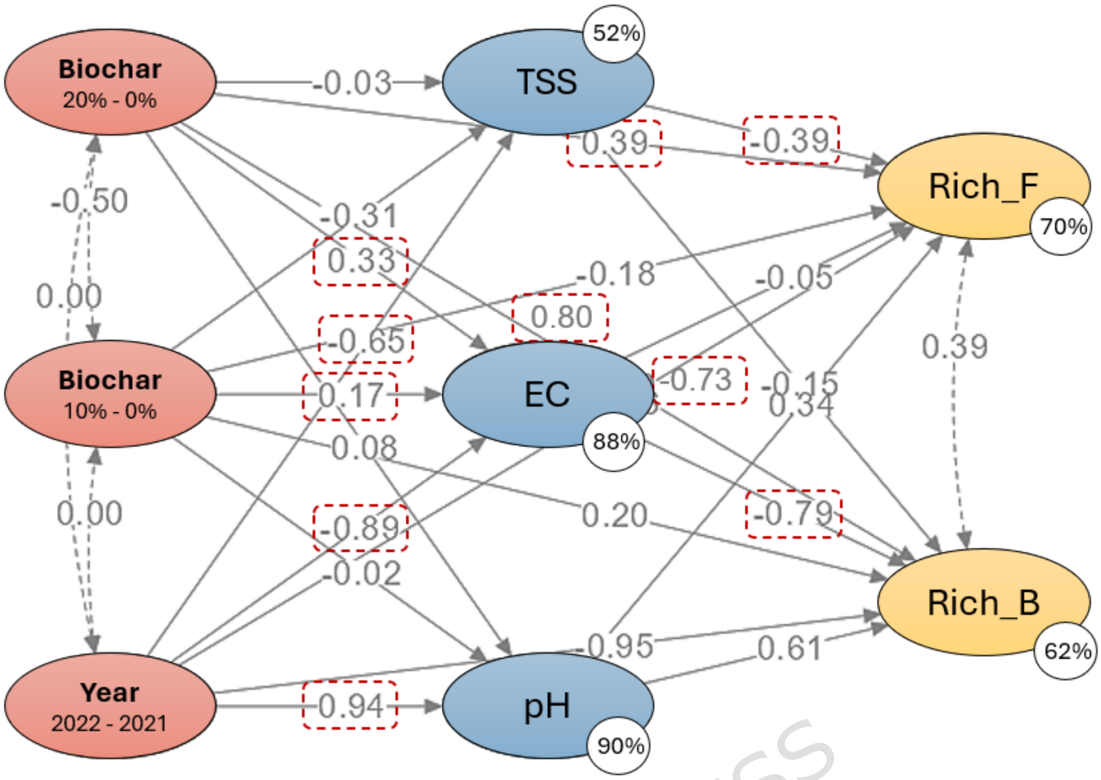


Fig. 9. Simplified path model (SEM) showing the relationships between experimental treatments (SSB dose and year), abiotic factors measured in runoff (pH, EC, TSS), and microbial richness (bacterial richness - Rich_B; fungal richness - Rich_F). Exogenous variables (SSB dose contrasts and year) are shown in red, endogenous mediating variables (pH, EC, TSS) are shown in blue, and endogenous response variables (bacterial and fungal richness) are shown in yellow. Solid arrows represent hypothesized causal pathways, with standardized regression coefficients (β) shown along each path. All estimated β values are displayed; statistically significant effects ($p \leq 0.05$) are highlighted with red dashed boxes. Dashed curved arrows indicate covariances between endogenous variables. $R^2$ values (% explained variance) are presented in white circles within each endogenous variable.



## Conclusions

In a long-term rooftop experiment, SSB amendment was shown to influence hydrological, biological, and ecological processes within extensive green roof substrates without compromising runoff quality. Over the 40-month monitoring period, SSB incorporation improved substrate physical properties and water retention capacity, contributing to reduced runoff generation, particularly during dry periods, and moderating physical changes associated with substrate aging. Despite its nutrient-rich origin, runoff nutrient concentrations exhibited initial increases followed by interannual stabilization, consistent with gradual nutrient release dynamics rather than sustained leaching.  Improved substrate moisture conditions were accompanied by higher vegetation cover in SSB-amended substrates compared to the control, without evidence of a strictly linear dose–response relationship. This finding highlights the importance of substrate design for maintaining green roof functionality under variable climatic conditions. Microbial analyses revealed that SSB application, especially at 20% v/v, was associated with increased bacterial and fungal richness. Structural equation modelling suggested that microbial diversity patterns were associated with long-term changes in runoff characteristics and interannual variability, pointing to a potential influence of indirect and system factors rather than the effects linked to a single variable.

Although the findings are based on a single rooftop location under temperate conditions, the extended monitoring provides long-term empirical insight into system responses of extensive green roofs amended with SSB, offering evidence relevant to the design and evaluation of sustainable rooftop substrate systems.

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**Author Contributions**

Data curation, M.P.; investigation, M.P., L.V., J.U., K.O., J.V., and V.F.; methodology, M.P., K.O., P.S., and V.F.; formal analysis, M.P., M.H., and P.S.; supervision, G.S.; validation, V.F.; writing original draft, M.P., K.O., and L.V. All authors have read and agreed to the published version of the manuscript.



**Competing interests**

The authors declare no competing interests.

**Data availability**

All data are available from the corresponding author upon request.

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