

Article

Optimization of Peat-Vermicompost Green Roof Substrates Through Biochar Additions

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Abstract

Replacing peat in green roof substrates with sustainable alternatives while maintaining plant performance and ecosystem services remains a critical challenge. We studied biochar-substrate interactions across four commercial green roof formulations (based on the type of organic component) in a greenhouse experiment: pure vermicompost, vermicompost + fen peat, fen peat, and mixed fen/high-moor peat. Substrates were amended with straw biochar, pine bark biochar, or left unamended (5% *v/v*, *n* = 4 replicates) and planted with a grass seed mixture mimicking early green roof establishment. Plant growth, nutrient contents (nitrate and phosphate contents), and microbial indicators (microbial biomass carbon (MBC), *q*CO₂, and enzyme activities) were measured 30 days after the experiment began. Straw biochar in vermicompost boosted nitrate (90.8 mg kg⁻¹) and root N (3.1%) compared to the control, while pine bark biochar in mixed peat released phosphate (+375%) and maximized MBC (874 μg g⁻¹). Biochar intensified substrate effects, suppressing CO₂ in peat through liming effects (pH from 4.6 to 6.5–7.1) but priming respiration in vermicompost via labile C supply. PCA explained 63% of the variance, with nitrate, plant N, and microbial parameters driving substrate separation. These short-term greenhouse results demonstrate critical biochar-substrate specificity for green roof substrate development, emphasizing formulation-specific matching over universal biochar application.

Keywords: biochar; peat substrates; vermicompost; green roofs; nutrient dynamics; microbial functioning; microbial respiration; substrate interactions; plant growth



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1. Introduction

Green roofs are implemented as a nature-based solution to mitigate urban environmental problems such as stormwater runoff, overheating of buildings, biodiversity loss in densely built areas, and to reduce the urban heat island effect [1,2]. The performance of green roofs and the ecosystem functions they can support strongly depend on the properties of the substrate, which control plant establishment, water storage, and nutrient cycling [3].

Green roof substrates are usually engineered mixtures of lightweight mineral components (including expanded clay, perlite, pumice, zeolite, crushed bricks), sand to create the appropriate air regime and organic amendments that improve water-holding capacity and nutrient supply (coconut fiber, peat, various composts) [3], and amendments from recycled materials such as biochar; sometimes the substrates are made based on the regional soil [4]. Optimal substrates must combine low bulk density with sufficient water

availability, aeration, and long-term structural stability to sustain vegetation under shallow rooting conditions and frequent droughts. However, conventional substrates often rely heavily on peat [4] as the main organic component because of its high water retention, low density, and availability in regions where green roofs are built. The use of peat, in turn, can increase CO₂ efflux from green roofs, prompting policies to phase out peat in horticulture and related applications. Thus, there is a demand for more sustainable organic materials that can partly or fully replace peat in green roof substrates without reducing plant performance.

Among potential organic components for sustainable green roof substrates, vermicompost has emerged as an attractive alternative to peat. Vermicompost differs substantially from peat in its high contents of plant-available nutrients (derived from mineralized organic matter), beneficial microbial communities (enriched by earthworm gut activity), and plant growth-promoting compounds, such as phytohormones (auxins, cytokinins, gibberellins) and enzymes produced during vermicomposting [4,5]. Experiments with the addition of green-waste compost to extensive green roofs have shown that 10% of the compost in commercial substrates (based on crushed brick) can improve the growth of *Allium schoenoprasum*, *Limonium latifolium*, *Melica ciliata*, and *Nepeta × faassenii* [6]. In another study with the application (rate 0, 20, 40, 60, 80, and 100%) of commercial compost produced from municipal yard waste to a heat-expanded shale and sand base for an intensive green roof, the maximum performance and yield of peppers was revealed for 60 or 80% addition [7]. At the same time, excessive vermicompost rates may increase nutrient leaching, indicating the need to optimize its proportion in green roof substrates. Although vermicompost and compost amendments have been shown to improve plant growth in green roofs, the optimal proportion and performance of vermicompost in substrate blends remains unclear, especially when the substrate must balance plant nutrition with nutrient leaching, water retention, and physical structure. Consequently, vermicompost-based and peat-based substrates represent contrasting substrate environments in which amendment effects may differ considerably.

Biochar has been tested as an amendment to green roof substrates [8]. Due to its porous structure and high specific surface area, biochar can increase water-holding capacity, decrease bulk density, and reduce nitrogen and phosphorus leaching from substrates [9] under intensive irrigation or rainfall. It was shown that the addition of green waste biochar to a Scoria-based substrate at 40 vol % increased the water-holding capacity, yielding a 16% increase in plant-available water at a 30% dosage, and a considerable reduction in bulk density [10]. The addition of mixed conifer wood biochar (25 t ha⁻¹) to a commercial green roof substrate composed of porous aggregate, composted organic matter, and fine sand enhanced plant growth and reproduction, and water retention capacity [11]. In contrast, the application of hardwood mixture biochar to a commercial substrate based on proprietary aggregate at 10 wt % did not have a considerable effect on water retention [12], indicating that the effect of biochar amendments varies across experiments and depends on the type of biochar added and the initial substrate. In addition to reducing bulk density and retaining nutrients [12], biochar can alter microbial communities and soil biochemical processes in green roof substrates [13]. Experimental studies with sludge-derived biochar (application rates of 5–20% to natural soil used as green roof substrate) increased the fungal biomass by 300–600% and of eukaryotes by 200–380%. Biochar additions also increased soil microbial taxon richness and altered the microbial community structure [13]. The general direction of changes observed after biochar addition to soil or substrate depends on the biochar type, substrate composition, and the plant species present [14]. The study comparing traditional peat-based substrates with mixtures of peat, vermicompost, and biochar in various proportions showed that geranium and petunia can be cultivated on

a peat-based substrate containing up to 12% of biochar and/or up to 30% vermicompost. Plants in these substrates showed a similar or enhanced physiological response to those grown in the control using commercial peat-based substrate [15]. However, the effects of interactive contrasts between biochar types and vermicompost-peat gradients on the early establishment of plant communities, nutrient dynamics, and microbial activity in substrates remain underexplored, particularly at low addition rates and across realistic green roof substrate compositions.

Recent studies focused on green roof substrates have two important knowledge gaps. First, most studies test either peat-based or mineral-dominated substrates with single organic additions, while systematic comparisons of vermicompost-based vs. peat-based mixtures under green roof conditions remain scarce. Second, although biochar is increasingly studied as a green roof amendment, experiments typically use one biochar type and focus on water retention, runoff quality, or plant yield [16], rather than simultaneously assessing plant and substrate traits. Only a few studies have considered biochars derived from different feedstocks in the context of green roofs [17], and these have mostly addressed digestate or wood-derived biochar rather than straw- or bark-based materials. Thus, the central question of the study is how different biochar feedstocks interact with contrasting green roof substrate mixtures ranging from vermicompost-dominated to peat-dominated systems.

This study first aimed to compare the effects of four substrate base compositions relevant to extensive green roofs in a temperate climate, ranging from vermicompost-dominated to peat-dominated mixtures, on plant growth under controlled greenhouse conditions. Second, we aimed to evaluate two contrasting biochar feedstocks (straw- and pine-bark-derived biochar) added at the same rate to each substrate base, allowing direct assessment of feedstock-dependent effects on substrate chemical and biological properties, which in turn could affect plant community development. Compared with previous studies, we made the first systematic factorial comparison of two contrasting biochars (straw vs. pine bark) across a complete vermicompost-to-peat substrate gradient. Additionally, we simultaneously quantify effects on plant establishment (grass mixture), nutrient mobilization (NO_3/PO_4), and microbial function (microbial biomass, CO_2 emissions, activities of C-cycle enzymes) under controlled conditions that mimic early green roof establishment.

The following hypotheses were tested: (i) Biochar effects on plant establishment depend on substrate composition, with peat- and vermicompost-based substrates differing in their biomass and carbon accumulation responses to biochar addition; (ii) straw-derived and pine-bark-derived biochar would have different impacts on nutrient availability, plant N uptake, and microbial activity due to their contrasting chemical composition and surface properties; (iii) Substrate composition is the primary driver of microbial biomass and activity, while biochar acts as a secondary modifier whose effects depend on the specific substrate–biochar combination.

2. Materials and Methods

2.1. Experimental Design

The experiment was conducted in 2025 at Tula State University. Twelve substrates were created for testing, and all components were taken by volume (Table 1). The dominant organic compound in the substrate was either vermicompost or fen peat, and mixtures having either vermicompost + fen peat or fen peat + high-moor peat were prepared (Table 1). These substrates were selected due to the commercial availability of components, low weight, and varying organic matter quality, with the C/N ratio increasing from vermicompost-based mixtures to fen- and high-moor peat-based mixtures (Table A1). In addition, substrates differed in the composition of their mineral fractions, which likely

influenced physical characteristics such as porosity, bulk density, and water retention and may have contributed to the observed biological responses. Therefore, the results that we present reflect integrated effects of full substrate formulations rather than the influence of organic matter composition alone. Biochar was added to enhance nutrient retention in the substrates. Four substrate mixtures were combined with three biochar treatments (control, straw biochar, pine bark biochar), yielding 12 treatment combinations, each replicated 4 times ($n = 48$ pots). Biochars were prepared from maize straw or pine bark (1–2 cm fraction) at 350 °C for 3 h. Raman spectra of biochar (Spectrometer EnSpectr M532, “Spektr-M LLC”, Chernogolovka, Russia) were acquired using a 532 nm excitation source with a laser current of 30 mA and a voltage of 4 V ($\approx 50\%$ laser power). The integration time was 10 s per acquisition, with 5 accumulations averaged for each spectrum. The detector gain was set to 1.3 with an offset of 2.542. Spectra were recorded over the range of approximately 120–3680 cm^{-1} . Cosmic ray removal and automatic baseline correction were applied during post-processing. No active cooling was used during measurements. Signal intensity was automatically adjusted, and background subtraction was enabled. The specific surface area of the biochar was determined using methylene blue [18]. The final concentration of adsorbed methylene blue was measured spectrophotometrically (590 nm, Aquilon SF-103 spectrophotometer, “Aquilon”, Podolsk, Russia). Specific surface area of straw-derived biochar was $230 \pm 10 \text{ m}^2 \text{ g}^{-1}$, and for the pine bark-derived biochar was $218 \pm 7 \text{ m}^2 \text{ g}^{-1}$.

Table 1. Composition of substrates used in the experiment. All substrates were taken by volume. S means substrate. B1 means biochar prepared from maize straw. B2 means biochar prepared from pine bark.

Substrate	Sand %	Perlite, %	Sawdust, %	Vermi-Compost, %	Expanded Clay, %	Fen Peat, %	High-Moor Peat, %	Biochar, %
S1	25	20	5	50	-	-	-	
S1B1	25	20	5	45	-	-	-	5
S1B2	25	20	5	45	-	-	-	5
S2	25	20	-	30	-	25	-	
S2B1	25	20	-	30	-	20	-	5
S2B2	25	20	-	30	-	20	-	5
S3	25	5	-	-	10	60	-	
S3B1	25	5	-	-	10	55	-	5
S3B2	25	5	-	-	10	55	-	5
S4	25	-	-	-	10	35	30	
S4B1	25	-	-	-	10	30	30	5
S4B2	25	-	-	-	10	30	30	5

Each substrate was placed in 1 L pots (diameter was 11.5 cm and height were 9.6 cm) and moistened to 60% of its maximum water-holding capacity. Each pot was supplemented with 2 g of seed from a mixture of 15% perennial ryegrass (*Lolium perenne*), 15% annual ryegrass (*Lolium multiflorum*), 25% wheatgrass (*Triticum aestivum*), 20% festulopium (*Festulolium hercynicum*), 15% meadow fescue (*Festuca pratensis*), and 10% red fescue (*Festuca rubra*). The grass seed mixture represents realistic early-stage green roof vegetation development, in which multiple species establish simultaneously from seed rain. These species were chosen to create a functionally diverse grass cover, namely: perennial and annual ryegrass provide rapid germination and early biomass accumulation; wheatgrass contributes strong rooting and stress tolerance; festulolium combines rapid establishment with improved adaptability; meadow fescue offers moderate vigor and persistence; and red fescue provides tolerance to low fertility and drought with strong belowground allocation. Although the component grasses differ in germination and early growth dynamics, these

differences were common to all treatments and therefore do not confound the treatment comparisons. The experiment was conducted in a greenhouse at 20 °C for 30 days with a 16 h light/8 h dark photoperiod. Supplemental lighting (high-pressure sodium lamps, $\sim 400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) was provided for 16 h daily to ensure a minimum daily light integral of $12\text{--}16 \text{ mol m}^{-2} \text{d}^{-1}$, typical for grass seedling growth under controlled conditions. Pots were arranged in a completely randomized design, and pots remained in fixed positions throughout the experiment. Watering was performed every 2 days using the balance method. After 30 days, destructive sampling was performed, and plant biomass was separated into belowground and aboveground biomass.

2.2. Analyses of Substrate and Plant Biomass Properties

The carbon (C) and nitrogen (N) contents of the organic components of the substrates and of plant biomass were measured using dry combustion (Vario Max CN analyzer, Elementar, Langenselbold, Germany). pH was measured in water (component:water ratio = 1:5) using a Hanna pH meter (Hanna Instruments, Vöhringen, Germany); the substrate's water-holding capacity was measured by weight, separately for each substrate [19]. The substrates were analyzed for ammonium nitrogen and nitrate content by spectroscopy [20] (Spectrophotometer, Akvilon 103, Podolsk, Russia), available phosphate content (CAL extraction, following spectroscopic measurements at 880 nm [21]) (Spectrophotometer, Akvilon 103, Podolsk, Russia). Microbial biomass was measured by the substrate-induced respiration method, and microbial respiration was measured by incubation for 5 days at 22 °C; the stable values were taken [22]. The metabolic quotient ($q\text{CO}_2$) was calculated as the ratio of respiration to microbial biomass to indicate stress conditions for microorganisms. Activities of cellulase and catalase were measured using spectrophotometric methods [23] (Spectrophotometer, Akvilon 103, Podolsk, Russia).

2.3. Statistical Analyses

Statistical analyses followed a structured approach: two-way ANOVA was used for hypothesis testing, planned contrasts were used to directly evaluate H1–H3, and PCA was applied as an exploratory tool to visualize overall patterns. All statistical analyses were performed using R software (version 4.3 R Core Team, 2024). The significance level was set at $\alpha = 0.05$ for all tests.

A two-way Analysis of Variance (ANOVA) was conducted for each response variable to evaluate the main effects of substrate type (four levels: vermicompost, vermi + fen peat, fen peat, mixed peat) and biochar treatment (three levels: control, straw biochar, pine bark biochar), as well as their interaction (Substrate $\times$ Biochar). ANOVA assumptions were assessed by examining model residuals. Normality of ANOVA residuals was tested using the Shapiro–Wilk test, and homogeneity of variance was assessed using Levene's test on residuals grouped by treatment combination. Box–Cox transformation was applied to the following response variables to meet the ANOVA assumptions: NH_4 ($\lambda = -0.3$), PO_4 ($\lambda = 0.1$), CO_2 ($\lambda = 1.4$), NO_3 ($\lambda = 0$), Cellulase activity ($\lambda = -0.3$). Welch's ANOVA following Games-Howell Post hoc Test was applied to Roots C, $q\text{CO}_2$, Catalase activity, and Shoots C because assumptions of the ANOVA model were still violated after transformation. For the variables where ANOVA assumptions were met after transformation and where no interaction (Substrate $\times$ Biochar) was revealed, the Tukey test was performed at $p < 0.05$. For the variables in which the interaction (Substrate $\times$ Biochar) was significant, interaction plots were generated to illustrate the significant interaction effects ($p < 0.01$). Intersecting lines indicate substrate-specific responses of biochars.

Following ANOVA, planned contrasts were used to test three a priori hypotheses without inflation of Type I error, as contrasts are orthogonal and hypothesis-driven. Con-

trasts were defined according to the experimental design, with 12 treatment combinations (4 substrates $\times$ 3 biochar treatments).

The following hypotheses were tested:

H1. *Biochar effects on plant biomass and carbon accumulation are substrate-type dependent, with stronger effects in peat-based substrates compared to vermicompost-based substrates. This was tested using three contrasts: (i) biochar effect (Control vs. biochar treatments combined) within vermicompost-based substrates (vermicompost and vermi + fen peat), (ii) biochar effect within peat-based substrates (fen peat and mixed peat), and (iii) interaction contrast to test whether the biochar effect differed between substrate types. Variables tested: shoot biomass, root biomass, shoot-to-root ratio, and root C content.*

H2. *Straw and pine bark biochars differ in their effects on nutrient availability, plant nitrogen uptake, and microbial activity due to their contrasting chemical composition and surface properties. This was tested using three contrasts: (i) straw vs. pine bark biochar in vermicompost-based substrates, (ii) straw vs. pine bark biochar in peat-based substrates, and (iii) overall straw vs. pine bark effect averaged across substrate types. Variables tested: NH_4 , NO_3 , PO_4 , root N content, MBC, and CO_2 .*

H3. *Vermicompost-based substrates have higher microbial biomass, respiration, and enzymatic activities than peat-based substrates, and biochar additions modulate these differences depending on substrate type and biochar source. This was tested using three contrasts: (i) substrate type effect (vermicompost-based vs. peat-based) in control treatments only, (ii) substrate type effect in biochar-amended treatments only, and (iii) interaction contrast testing whether biochar modulates the substrate type effect. Variables tested: MBC, CO_2 , $q\text{CO}_2$, catalase, and cellulase activities.*

For each contrast, the contrast estimate, standard error, t-statistic, degrees of freedom, and *p*-value were calculated using the mean squared error (MSE) from the ANOVA model. Contrast analysis was performed on the same data (original or transformed (Box–Cox transformation)) used for ANOVA to ensure residuals met statistical assumptions. The transformed variables were: shoot-to-root ratio, NO_3 , PO_4 , catalase, and cellulase activities and CO_2 . Significant contrasts ($p < 0.05$) were interpreted in the context of the respective hypotheses.

Principal Component Analysis (PCA) was performed on all 16 response variables to explore multivariate patterns, and determine whether substrates could be grouped by base type (vermicompost-based vs. peat-based) or biochar treatment, and identify which variables contributed most to the observed differences. Prior to PCA, all variables were standardized (z-score transformation: mean = 0 and standard deviation = 1) to account for different measurement scales and units. Data suitability for PCA was assessed using the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy (acceptable if $\text{KMO} > 0.6$) and Bartlett's test of sphericity (significant if $p < 0.05$, indicating sufficient correlation among variables). PCA was conducted using the `prcomp()` function in R (stats package) on centered and scaled data. The number of principal components (PCs) to retain was determined using a scree plot and the Kaiser criterion (eigenvalues > 1), typically explaining ~60–80% of the cumulative variance. Here, PC1 + PC2 explained 63%. Variable loadings on PC1 and PC2 were examined to identify the main contributors to each axis. PCA results were visualized using biplots, with samples colored by substrate type and shaped by biochar treatment to visualize grouping patterns. Confidence ellipses (95% confidence level) were added to biplots to show within-group variation. Statistical differences between groups on principal component scores were tested using t-tests (for substrate type on PC1) and

one-way ANOVA (for biochar treatment on PC2). PCA visualizations were created using the *ggplot2* packages in R.

3. Results

3.1. Main Effects of Substrate Type and Biochar on Plant, Nutrient, and Microbial Variables

Substrate properties primarily drove vegetation responses; species-specific traits (e.g., root morphology) were not measured as the focus was on substrate functionality for overall cover establishment. Two-way ANOVA revealed a dominant effect of substrates ($p < 0.001$ for all parameters). Biochar type affected biomass (Shoots and Roots, $p < 0.001$), plant nutrition (NO_3 and PO_4 , $p < 0.02$, pH), and enzymatic activity (Cellulase and Catalase, $p < 0.01$) and qCO_2 (Table 2).

Table 2. Results of ANOVA for the plant and substrate properties. F and p -values are shown. Cell is cellulase activity, Cat is catalase activity, W is water holding capacity, C is carbon, N is nitrogen, MBC is microbial biomass, qCO_2 is metabolic quotient.

Variable	Substrate		Biochar		Interaction	
	F	p	F	p	F	p
Shoots	165.62	<0.001	33.13	<0.001	4.61	0.001
RootsN	32.92	<0.001	0.29	0.75	3.73	0.005
Roots	74.03	<0.001	8.75	<0.001	1.13	0.364
RootsC	41.02	<0.001	2.6	0.088	0.84	0.55
ShootsC	138.4	<0.001	5.16	0.011	2.1	0.077
ShootsN	40.6	<0.001	9.01	<0.001	2.37	0.05
NO_3	163.23	<0.001	5.69	0.007	3.83	0.005
PO_4	12.2	<0.001	4.62	0.016	33.55	<0.001
pH	50.16	<0.001	450.62	<0.001	9.95	<0.001
W	226.03	<0.001	0.74	0.486	3.88	0.004
NH_4	39.46	<0.001	0.37	0.692	0.7	0.652
MBC	217.34	<0.001	0.37	0.692	5.56	<0.001
Cell	111.52	<0.001	24.87	<0.001	4.08	0.003
Cat	109.47	<0.001	6.25	0.005	3.91	0.004
qCO_2	52.16	<0.001	7.35	0.002	3.69	0.006
CO_2	38.5	<0.001	1.28	0.29	2.58	0.035

The highest root biomass was observed for plants grown on the vermicompost + fen peat substrates, and the lowest for pure vermicompost. Clear separation in root C content was observed between peat-based and vermicompost-based substrates, with higher values in the former. The minimal shoot C was measured for the vermicompost substrate, and the maximum for the fen peat. Shoot N was clearly separated between the vermicompost-based substrates, where maximal values were found, and the peat-based substrates (Figure 1).

Peat-based substrates contained more NH_4 than vermicompost-based substrates. Higher CO_2 emissions were found from the fen peat-substrates than for the other substrates, and biochar addition effects were substrate-specific (Figure 2).

Peat-based substrates had higher cellulase activity than vermicompost-based substrates, reflecting composition specificity. The metabolic quotient varied depending on the substrate $\times$ biochar interaction ($p = 0.006$). Mixed peat showed the lowest qCO_2 values, indicating high microbial respiration efficiency and correlating with increased microbial biomass. The greatest microbial community stress was observed in fen peat with pine bark biochar, where qCO_2 was 3.7 times higher than mixed peat and 70% higher than the fen peat control. The addition of straw biochar induced stress in the vermicompost + peat substrate, whereas the effect was minimal in vermicompost. Low qCO_2 values in mixed

peat indicated favorable conditions for microorganisms, while fen peat demonstrated sensitivity to biochar additives. The significant effects on shoot and root biomass are relevant to H1 (plant establishment), whereas the effects on NO_3 and PO_4 relate to H2 (nutrient dynamics). Differences in MBC, CO_2 emission, qCO_2 , and enzyme activities provide the basis for evaluating H3 (microbial functioning).

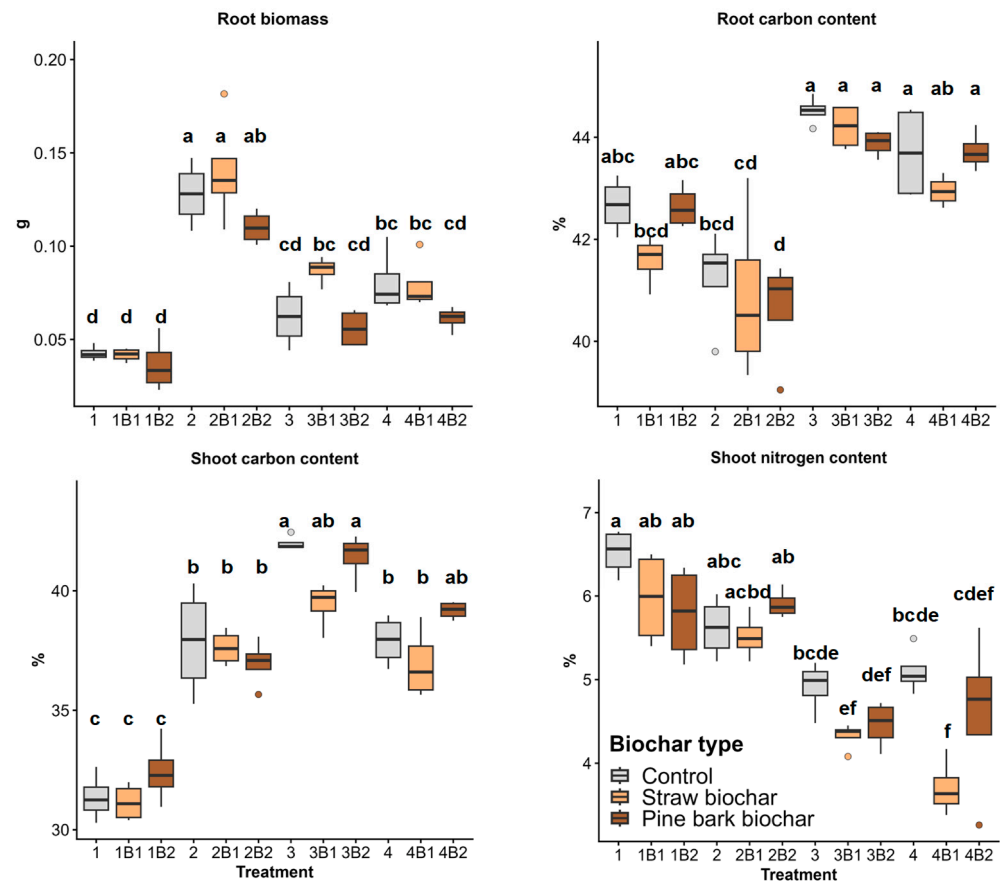


Figure 1. Root biomass, root and shoot carbon (C), and shoot nitrogen contents for the plants grown on vermicompost (1 (pure vermicompost) and 2 (vermicompost + fen peat)) and peat-based (3 (fen peat) and 4 (mixed peat)) substrates. B1 is straw-derived biochar and B2 is pine bark-derived biochar. Data are mean and st. error, $n = 4$. Letters are results of Tukey tests, $p < 0.05$. Original data are presented. The specific tests and transformation applied are written in the materials and method section. The colored circles that appear above and/or below the whiskers are the outliers.

3.2. Interaction of Biochar and Substrate to Modulate Plant Growth, Soil Properties and Microbial Activity

The interaction effect was significant for 11 of the 16 measured parameters ($p < 0.05$), with particularly strong effects for shoot biomass, phosphorus availability, and microbial biomass ($p < 0.001$), indicating pronounced substrate-specific responses to biochar (Table 2, Figure 1A).

Plant responses varied depending on the substrate–biochar combination (Figure 3). The strongest stimulation of shoot growth was observed in the vermicompost + fen peat substrate, irrespective of biochar addition, although straw biochar produced a slightly greater effect. In contrast, the effect of pine bark biochar on shoot biomass depended on the substrate type, yielding either positive or negative responses. Root N content was higher in vermicompost than in peat-based substrates, reaching a maximum in vermicompost + fen peat amended with pine bark biochar and a minimum in mixed peat amended with straw biochar.

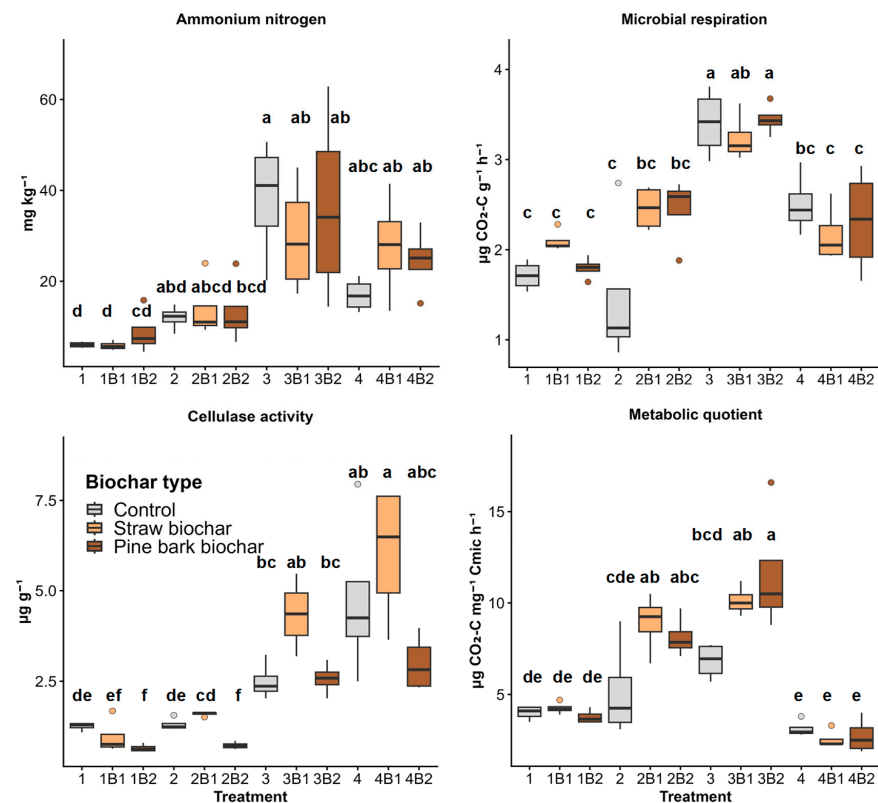


Figure 2. Ammonium nitrogen, microbial respiration, cellulase activity and metabolic quotient for the vermicompost (1 (pure vermicompost) and 2 (vermicompost + fen peat)) and peat-based (3 (fen peat) and 4 (mixed peat)) substrates. B1 is straw-derived biochar and B2 is pine bark-derived biochar. Data are mean and st. error, $n = 4$. Letters are results of Tukey tests, $p < 0.05$. Original data are presented. The specific tests and transformation applied are written in the materials and method section. The colored circles that appear above and/or below the whiskers are the outliers.

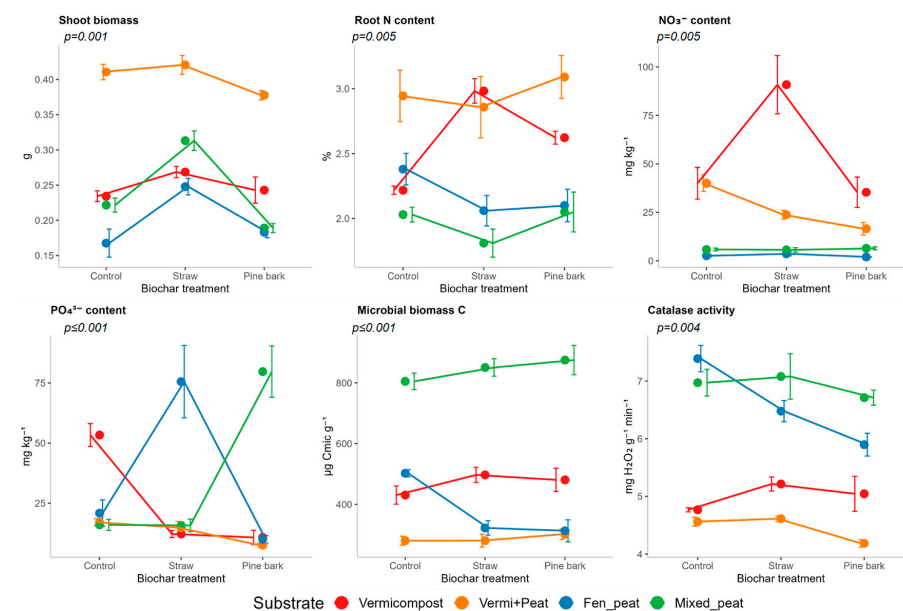


Figure 3. Substrate × Biochar type interactions for key traits from Table 2. Interaction effects: $p < 0.001$ (PO₄, MBC), $p = 0.001$ (Shoot biomass), $p \leq 0.005$ (others). Error bars show mean ± SE (n = 4). Original data are presented. N is nitrogen, C and carbon, NO₃⁻ is nitrate content in substrate, PO₄³⁻ is the available phosphorus content. Data are mean and st. error, $n = 4$.

Nutrient availability also showed clear substrate-specific responses. The highest NO_3 content was recorded in vermicompost amended with straw biochar, whereas pine bark biochar markedly reduced NO_3 concentrations in the vermicompost + fen peat substrate. In contrast, PO_4 availability was greatest in mixed peat amended with pine bark biochar, followed by fen peat amended with straw biochar, while the lowest values occurred in vermicompost-based substrates.

Microbial responses further demonstrated the importance of substrate–biochar matching. Mixed peat supported the highest microbial biomass among all substrate types, with pine bark biochar increasing microbial biomass by an additional 8% compared with straw biochar. Biochar additions increased MBC in vermicompost substrates but reduced it in fen peat. Catalase activity also responded differently depending on substrate composition: biochar stimulated the activity in vermicompost-based substrates, whereas contrasting responses were observed in peat-based substrates, particularly following straw biochar addition.

3.3. Hypothesis-Driven Contrasts and Multivariate Patterns

To directly evaluate hypotheses H1–H3, planned contrasts were performed on the variables identified as significant in the ANOVA. Contrast analysis revealed that peat-based substrates with biochar addition increased shoot biomass and the shoot/root ratio (H1b) (Table 3); also, shoot biomass in the biochar-added peat-based substrates was greater than in vermicompost-based substrates (H1c). The contents of NO_3 and PO_4 were higher in vermicompost-based substrates added with straw biochar than with pine bark (H2a) (Table 3). Overall, the substrates added with straw biochar had higher NO_3 and PO_4 contents than those added with pine bark-derived biochar (H2c). Microbial biomass, CO_2 emission, and activities of both enzymes were higher in peat-based substrate controls (without biochar addition) than in vermicompost-based controls (H3a) (Table 3). When only biochar-added substrates were compared (H3b), the same differences were found: peat-based substrates had higher parameters on the above named indicators than vermicompost-based substrates. When the effect of biochar addition into various substrates was compared (H3c), biochar in peat-based substrates effected more on MBC, CO_2 emission, and catalase activity than in vermicompost-based substrates. In contrast, the addition of biochar to vermicompost-based substrates had a greater effect on cellulase activity than on peat-based substrates.

Table 3. Significant contrasts ($p < 0.05$) from two-way ANOVA (Substrate $\times$ Biochar) testing hypothesis-driven comparisons on plant growth, nutrient availability, and microbial activity between biochar treatments and substrate types. Values represent differences in means according to contrast weights (e.g., H1b: peat-control vs. peat-biochar averaged across peat and mixed peat treatments for the control and biochar addition). The following transformations were conducted: Box–Cox–StoRn ($\lambda = -0.3$), P- PO_4 ($\lambda = 0.1$), CO_2 ($\lambda = 1.4$), Cat ($\lambda = -0.8$), Cell ($\lambda = -0.3$). Log-transformation: N- NO_3 . Other variables met ANOVA assumptions. StoR is shoot to root ratio, Cat is the catalase activity, Cell is cellulase activity, and MBC is microbial biomass carbon. Contrast interpretation: Positive estimate = first condition > second condition; negative estimate = second condition > first condition. Only significant contrasts are presented.

Contrast	Variable	Estimate-SE	t-Value	p-Value	Biological Meaning
Plant traits					
H1b: Peat-based control vs. peat-based biochar substrates	Shoots	−0.039 (0.010)	−3.72	<0.001	Biochar increased shoots biomass.

Table 3. Cont.

Contrast	Variable	Estimate-SE	t-Value	p-Value	Biological Meaning
H1b: Peat-based control vs. peat-based biochar substrates	StoR	−0.132 (0.057)	−2.32	0.026	Biochar increased StoR ratio.
	Shoots N	0.734 (0.191)	3.85	<0.001	Biochar addition decreased shoot N.
H1c: Interaction (biochar effect stronger in peat than in vermicompost substrates?)	Shoots	−0.017 (0.007)	−2.29	0.028	Biochar in vermicompost stimulated shoot growth.
Plant nutrition					
H2a: Vermicompost-based substrates added with straw vs. pine bark biochar	N-NO ₃	0.685 (0.181)	3.78	<0.001	Straw-derived biochar had stronger effect than pine bark on available nutrients.
	P-PO ₄	0.538 (0.234)	2.3	0.027	
H2c: Overall straw vs. pine bark biochar	N-NO ₃	0.423 (0.128)	3.3	0.002	
	P-PO ₄	0.386 (0.165)	2.34	0.025	
Microbial traits					
H3a: Vermicompost vs. peat-based substrates (only control)	Cat	−0.106 (0.008)	−13.34	<0.001	Peat substrates had stronger effect on microbial activity than vermicompost.
	Cell	−0.747 (0.101)	−7.37	<0.001	
	CO ₂	−1.879 (0.268)	−7	<0.001	
	MBC	−298.175 (28.665)	−10.4	<0.001	
H3b: Vermicompost vs. peat-based (only biochar-added)	Cat	−0.081 (0.006)	−14.49	<0.001	
	Cell	−1.194 (0.072)	−16.64	<0.001	
	CO ₂	−0.884 (0.190)	−4.66	<0.001	
	MBC	−200.250 (20.269)	−9.88	<0.001	
H3c: Interaction (Biochar modulates substrate effect? Comparison vermicompost vs. peat-based substrates)	Cat	−0.012 (0.005)	−2.53	0.016	Biochar in peat substrates stimulated catalase activity.
	Cell	0.223 (0.062)	3.59	<0.001	Biochar in vermicompost substrates stimulated cellulase activity.
	CO ₂	−0.498 (0.164)	−3.03	0.005	Biochar in peat substrates stimulated microbial activity.
	MBC	−48.963 (17.554)	−2.79	0.008	

PC1 and PC2 jointly explained 63% of the differences in substrate properties and their ability to support plant growth (Figure 4). The substrates were grouped by type, with PC1 separating vermicompost from peat-based substrates. Along the PC2, the substrates were separated into pure and mixed with other organic components. Substrates were not grouped by biochar addition, indicating that the substrate itself had a stronger effect on the properties than biochar addition did. PC1 was associated with N nutrition (RootsN, NO₃, ShootsN) and negatively with enzymes (Cat, Cell), reflecting N migration from soil to biomass. PC2 correlated with shoot C, root C, and microbial biomass, and was negatively correlated with growth, indicating resource redistribution. Grouping by substrates (vermicompost vs. peat) in PC1 confirmed the ANOVA results.

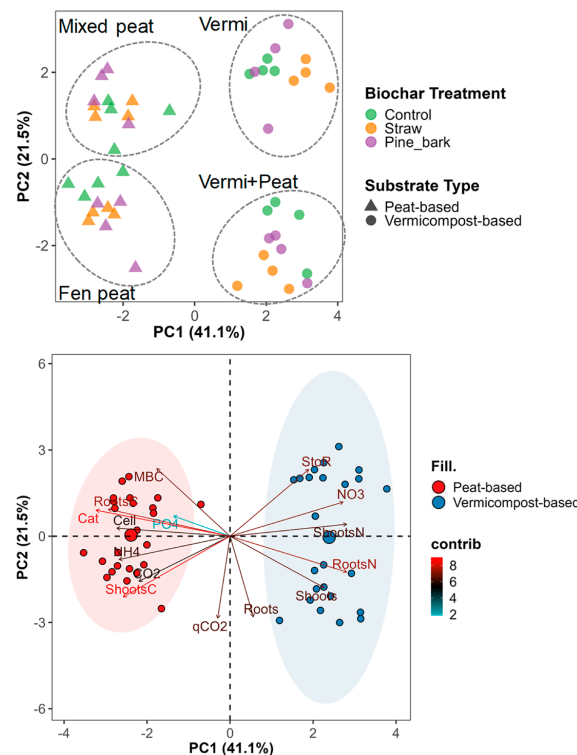


Figure 4. Principal component analysis (PCA) of substrate $\times$ biochar effects. PCA scores colored by biochar treatment (control, straw, pine bark), shapes by substrate type (**top**). Biplot with variable loadings (**bottom**). Ellipses = 95% confidence. Together PC1 + PC2 explain 62.6% of variance, $n = 48$. The contribution of factors (contrib) is provided by the color legend.

4. Discussion

The present study demonstrated that substrate type was the primary driver of plant, nutrient, and microbial responses, while biochar effects were largely dependent on substrate composition. Vermicompost-based substrates provided greater root growth and N for plant growth (Figure 1), which correlated with the PCA results. These observations could also be explained by increased mineralization of organic N in vermicompost, driven by active earthworms and enzymes [24]. However, these responses should not be attributed solely to the dominant organic component. The substrates also differed in mineral composition and associated physicochemical properties, which may have influenced nutrient availability, aeration, and water retention. Therefore, the observed plant responses reflect the combined effects of complete substrate composition rather than isolated effects of vermicompost or peat alone. The greater N availability observed in vermicompost-containing substrates is consistent with previous studies reporting enhanced plant growth and nutrient uptake following the incorporation of compost or vermicompost into green roof substrates. Similar positive effects of compost-based amendments on plant establishment have been reported for both extensive and intensive green roof systems [6,7].

In contrast, the mixed peat substrate had the highest MBC and metabolic efficiency values (Figures 2 and 3), which could reflect optimal moisture, aeration, and available C conditions for the microbial communities. The substrate-specific effect of pine bark biochar on PO_4 availability was particularly noticeable (Figure 3): mixed peat showed a sharp release of PO_4 (3.75 times higher than control) probably due to organic acids desorbing Al/Fe-P at an optimal pH of ~ 6.5 [8,25]. In the vermicompost substrate, the PO_4 decreased, possibly due to the high adsorption capacity of biochar and possible precipitation of PO_4 with Ca/Mg or organic compounds. Fen peat + straw biochar also showed the highest available phosphate values, likely due to mobilization processes and or original

PO₄ input with the biochar. This contrast suggested a possible dual role of biochar in our experiment: PO₄ mobilization from peat matrices vs. adsorption in organically rich environments. Similar substrate-specific effects were observed for NO₃ content: straw biochar increased NO₃ in vermicompost substrate. The same biochar, added to vermicompost + fen peat, caused metabolic stress, as indicated by qCO₂ values, and likely intensified competition for available N between plants and microorganisms. The PCA supported these patterns by showing a clear separation between vermicompost- and peat-based substrates, while the absence of distinct biochar clustering further emphasized the dominant role of substrate composition.

Plant responses were analyzed using planned contrasts (H1) to compare biochar effects within substrate types and to test differences between peat- and vermicompost-based substrates. Hypothesis H1b (on the effect of substrate and biochar on biomass/growth) was rejected, because the maximum of shoot biomass was reported for vermicompost + peat (with or without straw biochar addition), where the combination of high N (Roots N 3.1%) and improved aeration overcame the structural deficiency of pure vermicompost. H1c was confirmed, because the significant substrate-biochar interaction for shoot biomass demonstrated that the biochar effect (straw/pine bark) was stronger in peat substrates than in vermicompost, explaining 42% of the variability. Overall, H1 of planned contrasts was partially supported: biochar effects on plant biomass and carbon accumulation were clearly substrate-dependent, but the expected consistently stronger effects in peat-based substrates were not observed across all treatments.

Nutrient availability and plant uptake responses were analyzed using planned contrasts (H2) comparing straw and pine bark biochar across substrate types and their overall effects. The addition of straw biochar to vermicompost-based substrates increased NO₃ content compared to additions of bark biochar into peat substrates, which confirmed Hypothesis H2a. These results could be explained by the low adsorption of NH₄ on straw biochar, which increased organic nitrogen mineralization. Straw biochar had a greater overall effect on plant nutrition parameters, as supported by H2c: the maximum NO₃ content was observed in the vermicompost + straw biochar treatment. At the same time, the maximum PO₄ was found in mixed peat + pine bark biochar, which a pH shift upon biochar addition can explain. Straw biochar in vermicompost substrates increased pH from 5.5–6 (control) to 6.5–7.5, which is the optimal range for nitrogen mineralization processes. Pine bark biochar added to mixed substrates increased the pH to 6.5, which is more favorable for P cycling [26]. Its structure can explain these differences in the effect of biochar type: the Raman spectra of both biochars had characteristic D (~1350 cm⁻¹) and G (~1580 cm⁻¹) bands (Figure A2). A higher I_D/I_G ratio for the straw biochar (I_D/I_G ≈ 1.0–1.3) indicated an elevated degree of structural defects and the presence of oxygen-containing functional groups, which could enhance its sorption properties. However, this structure is more reactive and may have short-term toxic effects on microorganisms. In contrast, the pine bark biochar, with its lower I_D/I_G ratio (I_D/I_G ≈ 0.7–0.9), is characterized by a more ordered, graphite-like structure and fewer defects and functional groups, resulting in lower sorption activity but higher chemical stability and lower toxicity (Figure A2). Thus, H2 from planned contrasts was supported, as straw- and pine-bark-derived biochars showed distinct effects on nutrient availability and plant nutrient uptake across substrate types.

Microbial responses were analyzed using three planned contrasts defined in Hypothesis 3 (H3): (i) substrate type differences under control conditions, (ii) substrate type differences under biochar-amended conditions, and (iii) the interaction contrast testing whether biochar modifies substrate effects on microbial functioning. Results are presented according to these three contrasts. Cascading effects of substrate properties on microbial community functioning were revealed. MBC content in peat-based control substrates

was higher than those in vermicompost-based substrates (H3a), due to the presence of readily available organic matter in peat, which was also reflected in CO₂ emission values. Peat-based substrates with the addition of biochar showed higher MBC values than vermicompost-based substrates, when comparing pure substrates only (vermicompost vs. peat) and mixed substrates. Therefore, hypothesis H3b was not supported. These results are explained by a greater shift in the initial pH (Figure A1) of peat-based substrates and a higher content of available organic matter, which could stimulate microbial communities [27].

Interaction contrast showed that the effects of biochar depended on substrate type. CO₂ emission after the addition of biochar to peat substrates was suppressed. In contrast, it was stimulated for the vermicompost-based substrates, which could be attributed to the labile portion of C added with the biochar, thereby priming the decomposition of the original more available vermicompost substrates [28]. Cellulase activity was also higher in peat-based substrates added with straw biochar than in vermicompost substrates, reflecting accelerated substrate decomposition in the presence of more complex biochar or possible stimulation of microbial communities by metabolites contained in the biochar [29]. Previously, it was shown that straw biochar can increase fungal abundance, induce the production of ligninase and N-cycling enzymes [30], and, based on our results, probably also cellulase activity. In contrast, the suppression of cellulase activity was observed upon the addition of pine bark biochar, which is linked to compositional differences in biochar, such as aromaticity and C/N ratio [31]. An increase in qCO₂ suggested the development of stress conditions for the communities or altered microbial metabolic strategies. Similar qCO₂ patterns have been interpreted in the literature as shifts between more conservative (K-like) and fast-growing (r-like) microbial strategies [32].

Biochar had a stronger effect on microorganisms in peat substrates (H3c). qCO₂ in fen peat + pine bark biochar was higher compared to the control, whereas this indicator was 2.5 times lower in the vermicompost + straw treatment. These effects could be explained by the presence of toxic phenols/PAHs, especially in bark biochar, which could block microbial respiration in O₂-limited peat pores, although these compounds were not directly measured in this study. In vermicompost substrates, the presence of high organic matter with a narrow C:N ratio (i.e., high N content) may have mitigated the stress caused by biochar addition, since microorganisms can more readily utilize it. Pine bark biochar also reduced catalase activity in all substrates, most strongly in peat-based substrates. These results might be related to (1) phenolic compounds from lignin, which inhibit the Fe-center of the enzyme; (2) a sharp pH increase in acidic peat upon biochar addition (Figure A1); and (3) anaerobic microzones in biochar pores, which can also inhibit enzyme activity. Overall, H3 was partially supported: substrate type consistently influenced microbial biomass and activity, whereas the effects of biochar depended on substrate context and varied across microbial indicators.

These results provide initial evidence of substrate–biochar matching in green roof construction. Short-term testing suggests vermicompost supplemented with straw biochar may suit plant species requiring high N and root growth, delivering elevated NO₃ content and root N accumulation alongside stable microbial communities. Similarly, mixed peat with pine bark biochar at a pH of 6.5–7.1 appears promising for P-demanding species, supporting PO₄ release, microbial growth, and low qCO₂ indicative of metabolic efficiency, though PO₄ leaching risk exists in humid climates. However, fen peat + straw biochar warrants caution due to elevated CO₂ emissions, high qCO₂, which signals microbial stress, and reduced plant biomass observed here. These patterns from 30-day greenhouse testing require long-term field validation, including assessments of nutrient leaching, substrate stability, and green roof environmental exposure, to confirm practical suitability.

5. Conclusions

This study revealed strong substrate–biochar interactions controlling plant performance, nutrient availability, and microbial functioning in peat–vermicompost green roof systems. Vermicompost-based substrates combined with straw biochar promoted higher nitrogen availability, reflected in increased NO₃ supply and plant N uptake, whereas mixed peat amended with pine bark biochar enhanced phosphate availability and stimulated microbial biomass. Biochar effects were consistently substrate-dependent, with stronger responses in peat-based substrates than in vermicompost-based ones, particularly for nutrient dynamics and microbial activity. These patterns highlight the importance of substrate–biochar matching in regulating system functioning. The results are based on a 30-day greenhouse experiment and therefore reflect early establishment conditions rather than long-term ecosystem performance.

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Abbreviations

The following abbreviations are used in this manuscript:

C	Carbon
N	Nitrogen
PCA	Principal component analysis
ANOVA	Analysis of variance
Cat	Catalase activity
Cell	Cellulase activity
MBC	Microbial biomass carbon

Appendix A

Appendix A.1

Table A1. Carbon and nitrogen contents in the original components used for the substrate preparation. Data are mean and st. dev., n = 3.

Component	C, %	N, %
Vermicompost	22.0 ± 0.3	1.9 ± 0.04
Sawdust	47.6 ± 0.7	0.1 ± 0.05
Fen Peat	48.1 ± 1.9	1.1 ± 0.1
High Moor Peat	42.6 ± 0.8	0.8 ± 0.04
Straw-derived biochar	62.0 ± 1.5	1.5 ± 0.1
Pine bark-derived biochar	66.8 ± 1.6	0.5 ± 0.01

Appendix A.2

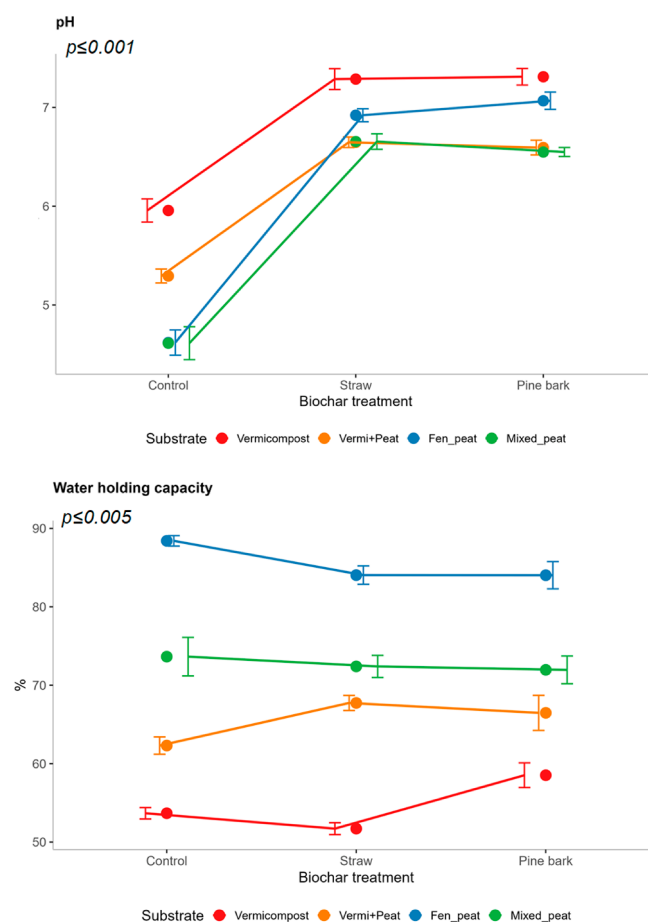


Figure A1. Substrate $\times$ Biochar type interactions for pH and water holding capacity. Interaction effects: Error bars show mean $\pm$ SE ($n = 4$).

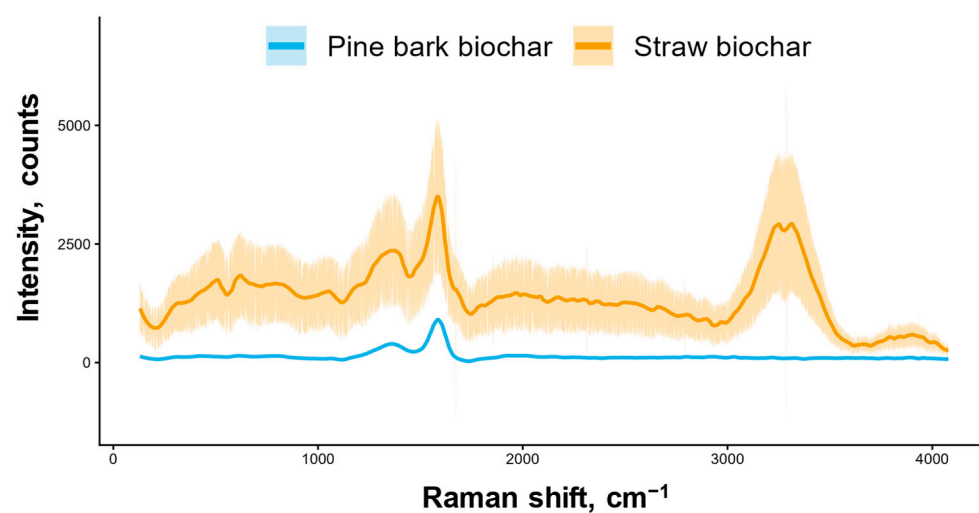


Figure A2. Raman spectra of biochar ($n = 3$). Raman spectra were recorded using a 532 nm laser (30 mA, 4 V), 10 s acquisition time, and 5 accumulations. Baseline correction and cosmic ray removal were applied.

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