

Article

Biochar and Compost as Sustainable Alternatives to Peat

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Abstract

The increasing demand for sustainable substrates in agriculture and urban greening calls for alternatives to peat, whose extraction poses significant environmental risks. This study assesses the potential of olive pomace biochar (OB), wood biochar (WB), and green compost (GC), alone or in combination, to partially replace peat in growing media and improve substrate properties and plant development. Ten different substrates were formulated by substituting 10–20% of a commercial peat-based substrate with these organic amendments, using the commercial substrate alone as a control. The effects of such replacements were evaluated in the following experiments: a germination test conducted in Petri dishes using four forage species (*Medicago polymorpha*, *Lolium perenne*, *Festuca arundinacea*, and *Lolium rigidum*); and two parallel pot experiments lasting 100 days each (one with *M. polymorpha* and *L. perenne*, and another with young *Olea Europaea* var. *Arbequina* saplings). This study evaluated the impact on plant development, as well as the physical properties and composition of the substrates during the incubation process. Germination and survival of forage species were comparable or improved in most treatments, except those including 20% OB, which consistently reduced germination—likely due to high electrical conductivity (>10dS/m). In the pot experiments, substrate pH and total carbon content increased significantly with biochar addition, particularly with 20% WB, which doubled total C relative to control. Both forage species (*Medicago polymorpha* and *Lolium perenne*) and the olive saplings (*Olea Europaea*) exhibited normal growth, with no significant differences in biomass, water content, or physiological stress indicators when compared to the control group. Nutrient uptake was found to be stable across treatments, although magnesium levels were below sufficiency thresholds without triggering visible deficiency symptoms. Overall, combining compost and biochar—particularly WB and GC—proved to be a viable strategy to reduce peat use while maintaining substrate quality and supporting robust plant growth. This approach proved effective across the different plant varieties tested, including *Medicago polymorpha*, *Lolium perenne*, and young olive plants, which together encompass a wide spectrum of agronomic and horticultural applications as well as contrasting growth and nutrient requirements. Adverse effects on early plant development can be avoided by carefully selecting and characterizing biochars, with specific attention to salinity and C/N ratio. This finding is crucial for the successful large-scale implementation of sustainable alternatives to peat.



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1. Introduction

The growing global demand for food and green infrastructure is intensifying pressure on natural resources and accelerating the need for more sustainable horticultural practices. In this context, the use of substrates derived from non-renewable resources, such as peat, has come under increasing scrutiny. Peatlands play a critical role in global carbon storage and biodiversity conservation, but their exploitation for horticulture and agriculture has led to severe environmental degradation, including ecosystem disruption, greenhouse gas emissions, and irreversible habitat loss [1]. As a result, peat extraction is progressively being regulated or banned in several regions, urging the development of renewable and eco-compatible alternatives for growing media.

Simultaneously, agro-industrial and urban green waste streams are expanding, often representing a poorly utilized resource with substantial environmental and economic costs. Valorizing these organic residues through composting or pyrolysis not only contributes to sustainable waste management but also aligns with circular economy principles and the European Green Deal's objectives [2,3]. Compost and biochar, the products of these transformation pathways, offer promising functional properties that can partially or fully replace peat in substrate formulations, hence their inclusion in the list of fertilizers and similar products under European regulations [4] is crucial to further promote their application. However, inherent variability arising from diverse feedstock, processing conditions, and final amendment properties demand careful evaluation of their effects on plant performance and substrate quality [5,6].

Biochar is a porous, carbon-rich material obtained by pyrolysis of biomass under limited oxygen. It is characterized by high chemical stability, a large specific surface area, and potential for improving nutrient retention, pH buffering, and water holding capacity (WHC) in growing media [7]. In contrast, compost provides plant-available nutrients, microbial inocula, and humified organic matter that can support plant growth and soil fertility [8]. The combination of biochar and compost may offer synergistic benefits, so the former enhances compost stability and mitigates nutrient leaching, while the latter improves the nutrient balance and biological activity of biochar-rich substrates [9].

Several studies have demonstrated the potential of biochar and compost as peat substitutes in horticultural substrates for a variety of crops including lettuce [10,11], tomato [12], marigold [13], cabbage [14], and rosemary [15], but despite broad evidence, much of this research focuses solely on the effects of peat substitution on just one plant species, either tomato [16,17] or blueberry [18]. This narrow focus presents a limitation, as different plant species have distinct requirements, meaning that variations in the physical and chemical properties of substrates can differentially affect their growth, as highlighted by Miler et al. [19]. Consequently, more comprehensive information is required on the viability of novel substrates, particularly those incorporating biochar, for broad application across diverse plant species. Nevertheless, the replacement level, biochar type, and interaction with compost significantly influence outcomes. High rates of biochar (above 30–50% *v/v*) have often been associated with adverse effects on germination and plant growth due to increased pH, salt accumulation, or nutrient imbalance [20]. Furthermore, biochar produced from certain feedstock—such as olive mill waste—can exhibit elevated electrical conductivity or phytotoxic compounds, requiring pretreatment or blending with other amendments to avoid detrimental effects on early plant development [21]. Compost quality

also varies with raw materials and maturation processes, affecting its compatibility with sensitive crops. Despite growing literature, knowledge remains limited on the suitability of specific combinations of compost and biochar for partial peat replacement in commercial substrates. This gap is particularly evident under Mediterranean conditions and in mixed plant systems involving both herbaceous and woody species. This study addresses such gap by evaluating the performance of two types of biochar—one derived from olive pomace (OB) and another from poplar wood (WB)—and a green compost (GC) produced from pruning residues and recycled wood chips, as partial substitutes for peat in a commercial growing medium. These materials were selected for their regional relevance and potential for upscaling within existing agri-food waste streams. Lastly, as the Mediterranean region is not a major global producer of peat, a significant portion of its consumed peat has to be imported, incurring in considerable transportation costs and higher prices [22,23]. In line with current sustainability goals, the study focuses on low to moderate substitution rates (10–20%) that could be realistically implemented in existing horticultural practices without requiring major infrastructure or economic shifts. Numerous studies have shown that applying extraordinarily high doses of compost or biochar may have adverse effects. For instance, Lazcano et al. [16] observed optimal aerial and root growth in plants with 10–20% compost substitutions, while concentrations above 50% proved detrimental. Similarly, García-Rodríguez et al. [11] reported that, while a 30% peat replacement with biochar could still improve plant growth, it delayed seed germination, and that higher concentrations negatively affected both growth and germination. Additionally, Bu et al. [24] demonstrated that 20% wood biochar was the most favorable proportion for plant growth, suggesting that substitution should not exceed 30%.

Here, the effects of peat substitution on the physical and chemical properties of the substrates are assessed, including pH, electrical conductivity, total carbon, total nitrogen, and water retention capacity. These parameters are critical to nutrient availability, microbial colonization, and root development, directly influencing plant establishment and productivity [25,26]. Moreover, this study examines the germination performance of four contrasting forage species—*Lolium rigidum*, *Lolium perenne*, *Festuca arundinacea*, and *Medicago polymorpha*—chosen for their agronomic value and differing sensitivities to substrate properties. These species also represent both monocots and dicots, enabling a broader generalization of findings and ensuring that the tested substrates meet the needs of diverse plant functional groups [27]. The selection of these specific species was based on their potential to thrive in challenging environments and their functional benefits (groundcover and cooling, drought and resilience, and substrate improvement), making them suitable for sustainable urban applications like green roofs, where one of the currently common components is peat. The potential use of *L. perenne* and *F. arundinacea* as groundcover in green roofs or green roof cooling due to their dense growth and high evapotranspiration rates have been demonstrated [28,29]. While *L. rigidum* has been considered as problematic for crops, it is also praised for its exceptional drought tolerance. This is a highly desirable trait for plants in water-limited environments like the Mediterranean region, or on extensive green roofs where irrigation is minimal. The combination of *M. polymorpha* as a N fixator in these growing media can improve their characteristics. *M. polymorpha* is a leguminous species known for its ability to form a symbiotic relationship with nitrogen-fixing bacteria, a particularly valuable trait for evaluating new growing media. By fixing atmospheric nitrogen, *M. polymorpha* can enrich the substrate and provide a natural nutrient source for itself and potentially other plants in the mix. This reduces the need for external fertilizers, enhancing the sustainability and long-term viability of the biochar-amended substrates. Therefore, the study of their combined response to the new substrates provides insights

into the potential for using resilient, sustainable, and low-maintenance species, as well as new media that will support beneficial plant-microbe interactions.

Additionally, young saplings of *Olea europaea* var. *Arbequina* were included to assess the suitability of these substrates for perennial woody crops. The global olive industry is undergoing a significant transformation, with traditional orchards being replaced by intensive and super-intensive cultivation systems. This shift demands a continuous supply of high-quality, certified plant material to meet regulatory standards. To produce the necessary volume of young olive trees, nursery propagation has become a critical stage. Consequently, there is an urgent need to develop and validate peat-free or peat-reduced growing media that can support robust root development in olive plantlets, while aligning with new sustainability standards and regulations. *Arbequina* is a widely cultivated olive variety in Mediterranean agriculture, known for its early productivity, high oil quality, and adaptation to super-intensive cultivation systems [30]. Given the economic and cultural importance of olive farming in the region, and its increasing integration with sustainable practices, understanding how biochar–compost substrates affect its early growth is highly relevant for growers and policy-makers alike. Previous works had characterized the individual performance of biochar and compost in soil-based systems but avoided an integrated, substrate-based cultivation scenario, which is more aligned with commercial nursery practices. As a major improvement, the current end-to-end experimental design now includes both germination and pot experiments under controlled greenhouse conditions, enabling a comprehensive evaluation of substrate performance from seedling establishment to early development.

The main objectives of the present work aim at: (i) characterizing the changes in key physicochemical properties of a commercial peat-based substrate when amended with low rates (10–20%) of OB, WB, and GC, alone or in combination; (ii) assessing the germination and early growth performance of annual forage species under these substrate conditions; and (iii) evaluating the physiological responses and nutrient status of olive saplings grown in peat-reduced media. By addressing these issues, this study will shape the development of more sustainable and locally adapted growing media that contribute to peat reduction targets while maintaining agronomic functionality. A key innovation of this work lies in its integrated assessment, combining an in-depth evaluation of soil physical and chemical properties with a comprehensive analysis of plant physiological status.

This study evaluates the functional performance of compost- and biochar-based alternatives to peat to advance the development of sustainable growing media. It further examines whether these materials could maintain substrate quality and plant performance while reducing peat dependence, and whether they could facilitate the incorporation of organic residues into circular, low-impact horticultural systems. The findings would be of interest to substrate producers, plant nurseries, and policymakers seeking the integration of organic waste into productive systems, to close nutrient and carbon loops and enhance the environmental performance of plant production systems.

2. Materials and Methods

The experimental protocol began with the selection and characterization of organic amendments to determine their suitability as partial peat replacements. The study was set up in two main stages: an initial assessment of substrate viability for seed germination, followed by the execution of two pot-based experiments designed to replicate long-term nursery growth conditions (Figure S1).

2.1. Substrates (Biochars, Compost and Commercial Substrate)

Two diverse biochars—compost and a commercial substrate, which was characterized by its richness in peat—were the original substrates used in this study.

Olive mill pomace was converted into biochar (hereafter OB—olive pomace biochar) within a rotatory cylindrical reactor (8 m long × 2 m diameter) operated by Carboliva S.L. (Puente del Obispo, Jaén, Spain). Slow pyrolysis was performed at 500 °C for 15 min under anoxic conditions, achieved by a continuous flux of N₂ and CO₂.

In addition, a wood biochar (hereinafter referred to as WB) produced from poplar wood in a fixed bed reactor with a residence time of 30 min. and a pyrolysis temperature of 520 °C was also used for comparative purposes.

Green compost (onwards GC) was produced by a traditional composting process in open piles and aerated by turning it. The raw material used for the composting stage was a mixture of pine wood chips from discarded pallets and garden pruning waste. The compost was supplied by Fertilizantes Orgánicos Melguizo S.L. (located in Los Palacios y Villafranca, Spain).

The commercial substrate was purchased from COMPO Iberia S.L. (Barcelona, Spain) and consisted of a blend of blond peat (35%), coconut fiber (50%), and perlite (15%). The characterization of these substrates is shown in Table 1. More information about OB and GC can be found in De la Rosa et al. [31]. More details about composting process and facilities are described in López et al. [32].

Table 1. Characterization of original substrates.

	TN (%)	TC (%)	Density (g ml ⁻¹)	WHC (%)	EC (μS cm ⁻¹)	pH (H ₂ O)
OB	1.13 ± 0.03	56.3 ± 1.7	0.57	78 ± 15	13,700 ± 389	9.9 ± 0.1
WB	0.22 ± 0.01	83.4 ± 0.2	0.50	159 ± 19	232 ± 48	9.5 ± 0.2
GC	0.68 ± 0.02	14.9 ± 0.2	0.75	114 ± 11	1184 ± 178	8.3 ± 0.2
PEAT	0.90 ± 0.02	41.3 ± 0.5	0.23	421 ± 38	1678 ± 190	4.9 ± 0.1

OB: olive pomace biochar; WB: wood biochar; GC: green compost; PEAT: commercial substrate. TN: total nitrogen content; TC: total carbon content; WHC: water holding capacity; EC: electrical conductivity.

For germination and pot experiments, 11 diverse treatments were prepared by mixing different amounts of said substrates. The control treatment was exclusively composed of commercial substrate rich in peat, whereas the other 10 treatments were formulated by combining peat with biochar and/or compost as illustrated in Figure 1. The components were mixed in a 100 L semi-automatic mixer at Plantas Continental S.A. company (Posadas, Córdoba, Spain) to ensure homogeneity.

	OB	WB	GC	PEAT
Control	0	0	0	100
OB_10	10	0	0	90
WB_10	0	10	0	90
GC_10	0	0	10	90
OB_5+GC_5	5	0	5	90
WB_5+GC_5	0	5	5	90
OB_20	20	0	0	80
WB_20	0	20	0	80
GC_20	0	0	20	80
OB_10+GC_10	10	0	10	80
WB_10+GC_10	0	10	10	80

Figure 1. Dry weight percentage of each organic amendment included in the substrate formulation for the 11 treatments used in the germination test and both pot experiments. Color intensity in the figure correlates directly with the increasing weight percentage of the component. OB: olive pomace biochar; WB: wood biochar; GC: green compost; PEAT: commercial substrate.

2.2. Germination Tests

Certified seed lots of *Lolium rigidum* (LR), *Medicago polymorpha* (MP), *Lolium perenne* (LP), and *Festuca arundinacea* (FA) were purchased from Semillas Silvestres S.L. (Córdoba, Spain) for germination experiment.

For the germination experiment, 7 g of substrate combination were placed in 10 cm diameter Petri dishes. The same species and quantity of seeds were used in a control Petri dish covered with humid filter paper to confirm seed viability. Nine seeds, activated at 4 °C during 48 h (process known as cold stratification [27]), were sown in every Petri dish. This procedure was repeated for every treatment. Petri dishes were then incubated in a growth chamber maintained at 24 °C and 40% relative humidity for 16 days. Number of germinated seeds were determined 8 and 16 days after sowing and germination rates were calculated.

2.3. Pot Experiments

Two parallel pot experiments were designed to meet the objectives of this study: understanding the effects of peat substitution by biochar and compost on forage crops and fruit crop (olive orchard).

1 L-pots were prepared for each treatment at Plantas Continental S.A. (Posadas, Córdoba, Spain). The experiments lasted 100 days and pots positions were randomized and rotated every two weeks to ensure homogeneity of conditions at the greenhouse. During the experiment, the greenhouse maintained a 12 h photoperiod, with constant temperature of 25 ± 3 °C, and the relative humidity was $60 \pm 10\%$. These conditions were monitored using an EL-1-USB Data-logger (Lascar Electronics Inc., Erie, PA, USA). Pots were watered every 2–3 days to maintain substrate humidity above 25% of WHC. In order to monitor potential pest infestations, yellow sticky traps were placed among the pots. Neither pesticides nor extra fertilizers were applied during these experiments.

2.3.1. Analysis of Substrates Properties

The following methods were used to analyze the physical properties and elemental composition of the growing media:

The pH was measured with a Crison 40 pH meter (Crison Instruments SA, Barcelona, Spain) in a 1:5 (*w/w*) substrate-distilled water mixture after 30 min shaking followed by 30 min of resting.

Substrate moisture (%) was measured in pots using a soil moisture meter PMS710 (Tsingtao Toky Instruments Co., Ltd., Qingdao, China). At the end of the experiment, total moisture (%) was determined by weight difference after drying the samples at 105 °C for 24 h in order to calculate the total water content.

Total carbon (TC) and total nitrogen (TN) contents of substrates and plant materials were determined using a Flash 2000 HT elemental micro-analyzer (Thermo Scientific, Bremen, Germany) equipped with a thermal conductivity detector. Dry combustion at 1020 °C converts all C to CO₂ and N to NO₂.

2.3.2. Pot Experiment I

Plant material selected for the first pot experiment consisted of: *Medicago polymorpha* selected for its interest as atmospheric N-fixer when in symbiosis with *Rhizobium*, and *Lolium perenne* chosen for its good results in preliminary germination tests.

1 L capacity pots (10 cm × 10 cm × 10 cm) were filled with 500 g of the substrate mixtures previously mentioned in Figure 1. In 3 pots of each substrate mixture, 9 seeds of *L. perenne* were sown, corresponding to a sowing density of 10 kg ha⁻¹. Similarly, 9 seeds of *M. polymorpha* were sown in 3 pots of each substrate mixture, according to a planting

density of 20 kg ha⁻¹. Control pots containing only substrate were included to monitor incubation effects.

Germination rates of *M. polymorpha* and *L. perenne* were counted weekly, and seedling survival was determined similarly starting after 7 weeks (49 days after sowing—49 DAS). Fresh weight of *M. polymorpha* and *L. perenne* was determined immediately after harvest (100 DAS), whereas dry weight was determined after drying 72 h at 60 °C. Their water content was calculated by difference in the fresh-to-dry weight.

Physical and chemical properties, and elemental composition of substrates were analyzed, as mentioned in Section 2.3.1, at the beginning and end of the 100-day experiment, immediately following plant harvest.

2.3.3. Pot Experiment II

For the second pot experiment, *Arbequina* olive variety (*Olea europaea* L.) was selected. This variety is widely cultivated across Mediterranean regions due to its capability for being drought resistant and its high productivity in super intensive olive grove [33]. Thus, semi-hardwood cuttings of *Arbequina* olive variety were planted in 1 L-pots containing 500 g of substrate of each treatment (8 replicates).

At 100 days after sowing (100 DAS) the physiological status of olive plants was assessed by measuring leaf Soil–Plant Analysis Development (SPAD) index and quantum yield. These measurements were taken on three photosynthetically active and fully expanded leaves in middle section (Ms) and in upper section of the plant, with eight plants per treatment. Measurements were recorded both before and after final irrigation. SPAD measurements were conducted using a portable SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL, USA). Quantum yield (QYPSII) measurements were conducted using a portable fluorimeter (FluorPen FP-100; Photon System Instruments, Brno, Czech Republic) in order to determine the efficiency of the photosystem II, as explained in García-Rodríguez et al. [11].

Plant height was measured immediately prior to harvest. Following harvest, the fresh weight of leaves and stems (manually separated) was determined. Dry weight was determined after drying at 60 °C for 72 h. In this dry plant tissues, total content of the following nutrients: Ca, K, Mg, Na, P, S, B, Cu, Fe, Mn, and Zn were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Varian Inc., ICP 720-ES, Palo Alto, CA, USA). For that, digestion of dry plant material with ultrapure nitric acid (Sigma-Aldrich, Burlington, MA, USA) was performed in a DigiPREP Block Digestion Systems (SCP Science, Quebec, QC, Canada).

Physical and chemical properties, and elemental composition of substrates were analyzed, as mentioned in Section 2.3.1, at the beginning and end of the 100-day experiment, immediately following plant harvest.

2.4. Statistical Analyses

Normality and homoscedasticity of data were assessed by Shapiro–Wilk and Levene tests, respectively. For normally distributed data, one-way ANOVA followed by HSD Tukey ($p \leq 0.05$) was conducted as post hoc analysis. Kruskal–Wallis and Mann–Whitney U tests were performed for variables that did not meet the assumptions. All statistical analyses were implemented using IBM SPSS Statistics 29.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Germination Performance Under Peat-Replaced Substrates

3.1.1. Germination Rates in Petri Dishes

Figure 2 illustrates the germination performance of four forage species (*Lolium rigidum*, *Medicago polymorpha*, *Lolium perenne*, and *Festuca arundinacea*) after 8 and 16 days of incubation in Petri dishes containing substrates amended with 10–20% biochars (OB; WB), and green compost (GC), either alone or in combination. The commercial peat-based substrate served as control. Germination of *L. rigidum* was enhanced in treatments amended with 10% WB or 10% GC, showing significantly greater germination percentages than the control. In contrast, *M. polymorpha* displayed the highest germination rate in the control treatment, while OB treatments consistently resulted in reduced germination. This trend suggests a possible inhibitory effect of OB affecting *M. polymorpha*, likely due to the high electrical conductivity of this biochar. In *L. perenne*, germination improved across most treatments, particularly with 10% WB and combined WB + GC treatments, except when OB was used alone at 10% or 20%, which significantly suppressed germination. Gascó et al. [34] also reported trends of increasing germination rates of *L. perenne* when peat was replaced by two biochars, but their effect was not significant. *F. arundinacea* responded positively to WB-based treatments, with the highest germination observed in the 10% and 20% WB treatments and the WB + GC combination. Its germination was also increased when 5% of WB was combined with 5% of GC.

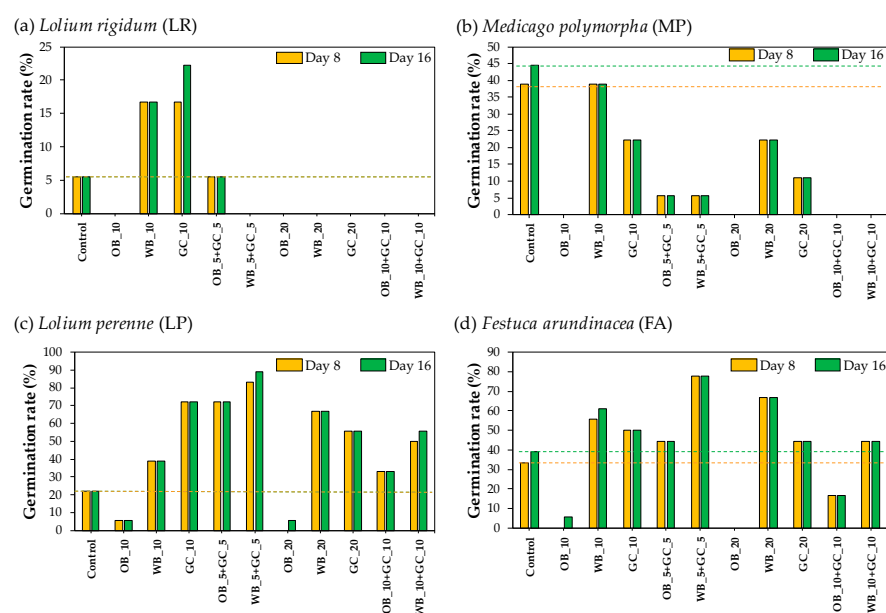


Figure 2. Germination rates (%) of (a) *Lolium rigidum* [LR], (b) *Medicago polymorpha* [MP], (c) *Lolium perenne* [LP] and (d) *Festuca arundinacea* [FA] in Petri dishes. Horizontal colored-lines indicate values for control treatment.

Germination rates differed significantly among the plant species studied. Highest germination percentages were observed for *L. perenne* and *F. arundinacea*, while rates remained below 25% and 45% for *L. rigidum* and *M. polymorpha*, respectively. However, all plant species showed germination rates lower than 45% for the commercial substrate. *F. arundinacea* showed germination rates between 30 and 40% at 8 and 16 DAS in the commercial substrate. Jung et al. [35] also found *F. arundinacea* germination rates around 40% when using a substrate with 30% of peat and 10% of perlite. These rates increased significantly to 80–90% when an exclusively peat-based substrate was used. The consistently low germination rate for *L. rigidum* across all treatments, including the control, suggests

an inherent issue with the seeds. Despite experimental conditions meeting the species' reported optimal range (25 °C with 12 h of light), the observed rates were low (<25%). This outcome is likely due to seed dormancy, a common characteristic of *L. rigidum* [36,37]. The brief cold pre-treatment (4 °C for 48 h) was likely insufficient to break this dormancy, as more extended periods of cold stratification (typically weeks) are often required [38,39]. Moreover, burial depth of seeds has been demonstrated to significantly impact *L. rigidum* germination, being optimum from 1 to 5 cm [36]; a depth higher than achieved in the Petri dishes. For *M. polymorpha*, germination was also notably low in most treatments (<45%). This pattern could be explained by a physical dormancy of these seeds [40]. Across all species, OB treatments—especially at 20%—were associated with the lowest germination percentages, reinforcing the need for caution when using this amendment without prior conditioning (e.g., leaching or dilution). This biochar was previously tested for complete grown olives in an olive orchard, proving better water and photosynthetic conditions of olives and improvement of soil properties [31] and also showing really high EC affecting seeds at germination stage. Elevated EC levels are widely reported to reduce germination rates across several species. For instance, Lin et al. [41] described reduced germination in *Lolium perenne* L. with increasing salinity. Although *Medicago polymorpha* has been shown to exhibit greater salinity tolerance compared to other species [42], it is still susceptible to inhibition at high EC concentrations. Probably, OB may be used after a washing process. In contrast, WB and GC demonstrated a generally neutral or positive influence on germination, indicating their potential suitability as partial peat substitutes in seedling production systems.

3.1.2. Evaluation of *Lolium perenne* and *Medicago polymorpha* Germination in Container-Grown Substrates

Germination of *Medicago polymorpha* was initially unaffected by substrate amendments, with rates at 16 DAS comparable to the control (Figure 3). From 20 DAS onward, seedling survival increased, particularly with 10% GC, while the 20% OB treatment consistently showed the lowest establishment, likely due to its high electrical conductivity [43]. Important differences were found between OB-20, OB_10 + GC_10 and WB_10 + GC_10 compared to GC_10, as similarly shown in the previous germination test. Noteworthy, green compost applied at 10 percent presented a greater survival rate during the experiment than at 20%. *Lolium perenne* exhibited delayed germination across all treatments, remaining below 20% until 60 DAS. García-Rodríguez et al. [11] demonstrated that germination can be delayed when treatments like these are used. However, from 70 DAS, seedling survival stabilized in most treatments, except in OB_20, where establishment remained limited. It is important to consider the differences in *L. perenne* germination observed in the Petri dish tests (Figure 2). For the control treatment, germination was lower at 8 and 16 days, but by 70 DAS of the pot experiment (Figure 3), it became similar to the other treatments. Even though the OB_10 treatment had a comparable final germination and survival rate from 70 DAS onward, its initial germination and survival in pot experiment (up to 65 DAS) was as low as in the first germination test. Additionally, OB_20 showed lower germination than 10% after 16 days in the germination Petri dish test. Phytotoxicity of OB, as previously mentioned, is related to its high EC. El Moussaoui et al. [44] also found phytotoxic effects on lettuce when using an olive pomace biochar due to its high EC (8770 $\mu\text{S cm}^{-1}$). There are plant species more tolerant to salinity: for instance, barley growth was unaffected by this reason. Notably, substrates containing WB or GC maintained similar seedling development to the commercial peat substrate. Since the most significant differences were found between GC_10 and OB_20, results highlight the relevance of amendment type and dosage, as high salinity or imbalanced nutrient profiles can compromise early plant establishment despite adequate carbon inputs [43].

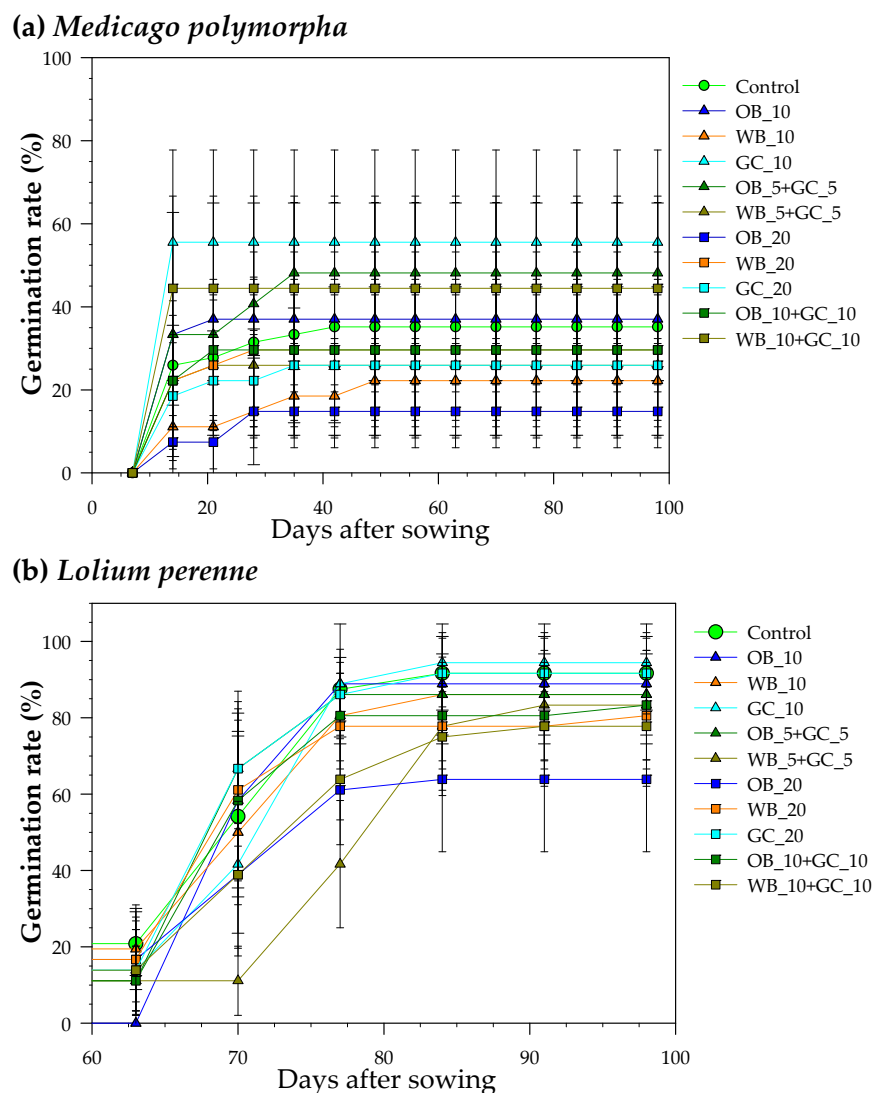


Figure 3. Germination rate (0–49 days after sowing) and seedling survival (49–100 days after sowing) of *Medicago polymorpha* (a) and *Lolium perenne* (b) during pot experiment. The green circle symbol denotes the control (commercial peat substrate). Triangles represent treatments with 10% substitution of peat, and squares represent treatments with 20% peat substitution.

3.2. Physical and Chemical Changes in Substrate Mixtures Following Peat Substitution

Partial substitution of commercial peat with biochar and compost amendments led to noticeable modifications in substrate physical and chemical properties (Table 2). As expected, pH increased across all treatments, both in planted and unplanted pots, reflecting the higher intrinsic pH of OB and WB (Table 1).

The application of WB at 10% and 20% significantly enhanced TC content compared to the control. This pattern persisted both in the initial mixtures and after 100 days of incubation across unplanted substrates and pots containing *Medicago polymorpha* and *Lolium perenne*. OB showed similar behavior. These outcomes align with previous reports highlighting biochar's high carbon content and chemical recalcitrance due to aromatic structures, which contribute to its persistence and potential for carbon sequestration [45,46]. The present findings also corroborate those reported by Picca et al. [47], who noted an increase in carbon content with biochar application aimed at peat reduction in a growing substrate within an analogous, extended-duration experiment (24 months). Conversely, substrates amended with 10% or 20% GC, alone or in combination with biochar, exhibited

reduced TC values after incubation. Moreover, the addition of GC at 5% combined with the studied biochars also led to a decreased total carbon content after 100 DAS.

Table 2. Physical and chemical properties and elemental composition of substrates (pure peat and mixtures) at the beginning and at the end of the experiments I and II.

	Treatment	Control Pots Without Plants		Experiment I		Experiment II	
		(0 DAS) ¹ Original	(100 DAS) Control Pots	(100 DAS)		(100 DAS) Olive Pots	
				MP ² -Pots	LP ³ -Pots		
pH	Control	5.0 ± 0.0	5.4 ± 0.0 ^f	5.5 ± 0.0 ^e	5.5 ± 0.0 ^{eb}	5.6 ± 0.0 ^h	
	OB_10	7.3 ± 0.1	6.2 ± 0.0 ^{cd}	6.3 ± 0.0 ^a	6.6 ± 0.0 ^{ab}	6.0 ± 0.0 ^d	
	WB_10	6.6 ± 0.0	6.6 ± 0.3 ^b	5.6 ± 0.3 ^d	6.2 ± 0.3 ^{db}	5.9 ± 0.0 ^e	
	GC_10	6.2 ± 0.0	6.1 ± 0.1 ^{de}	5.7 ± 0.1 ^d	6.0 ± 0.1 ^{db}	5.5 ± 0.0 ^h	
	OB_5 + GC_5	6.2 ± 0.0	5.9 ± 0.1 ^e	5.8 ± 0.1 ^c	6.0 ± 0.1 ^{cb}	5.8 ± 0.0 ^{fg}	
	WB_5 + GC_5	6.2 ± 0.0	5.6 ± 0.0 ^f	5.7 ± 0.0 ^d	5.8 ± 0.0 ^{db}	5.8 ± 0.0 ^f	
	OB_20	6.0 ± 0.0	7.1 ± 0.1 ^a	5.7 ± 0.1 ^d	6.6 ± 0.1 ^{db}	7.0 ± 0.1 ^a	
	WB_20	5.7 ± 0.0	6.2 ± 0.1 ^{cd}	5.8 ± 0.1 ^c	6.3 ± 0.1 ^{cb}	6.2 ± 0.0 ^c	
	GC_20	5.8 ± 0.1	6.0 ± 0.0 ^{de}	5.8 ± 0.0 ^c	6.2 ± 0.0 ^{cb}	6.2 ± 0.0 ^c	
	OB_10 + GC_10	6.0 ± 0.0	6.4 ± 0.0 ^c	6.3 ± 0.0 ^a	6.5 ± 0.0 ^{ab}	6.5 ± 0.0 ^b	
	WB_10 + GC_10	5.3 ± 0.0	5.5 ± 0.1 ^f	5.9 ± 0.1 ^b	6.0 ± 0.1 ^{cb}	5.7 ± 0.0 ^g	
	TC ⁴ (g kg ⁻¹)	Control	385 ± 22 ^e	446 ± 0 ^c	429 ± 2 ^{de}	418 ± 2 ^f	444 ± 2 ^d
		OB_10	432 ± 11 ^c	479 ± 3 ^b	460 ± 7 ^c	453 ± 1 ^d	431 ± 6 ^e
WB_10		438 ± 11 ^c	485 ± 6 ^b	586 ± 6 ^a	513 ± 1 ^b	532 ± 4 ^a	
GC_10		363 ± 33 ^f	380 ± 5 ^e	441 ± 4 ^d	389 ± 3 ^h	428 ± 5 ^e	
OB_5 + GC_5		412 ± 22 ^d	421 ± 0 ^d	417 ± 2 ^e	407 ± 2 ^g	450 ± 1 ^{cd}	
WB_5 + GC_5		440 ± 0 ^c	428 ± 1 ^d	490 ± 6 ^b	430 ± 0 ^e	457 ± 1 ^c	
OB_20		485 ± 22 ^b	447 ± 3 ^c	497 ± 2 ^b	480 ± 2 ^c	501 ± 0 ^b	
WB_20		506 ± 0 ^a	554 ± 4 ^a	578 ± 5 ^a	582 ± 0 ^a	537 ± 4 ^a	
GC_20		343 ± 55 ^g	375 ± 11 ^e	382 ± 7 ^f	375 ± 5 ⁱ	380 ± 0 ^g	
OB_10 + GC_10		384 ± 44 ^e	436 ± 1 ^{cd}	423 ± 1 ^e	451 ± 4 ^d	416 ± 5 ^f	
WB_10 + GC_10		451 ± 33 ^c	429 ± 2 ^d	489 ± 0 ^b	483 ± 3 ^c	461 ± 2 ^c	
TN ⁵ (g kg ⁻¹)		Control	7.9 ± 3.1	13.8 ± 1.0	14.0 ± 0.3	9.1 ± 1.6	18.6 ± 1.1
		OB_10	13.4 ± 3.7	15.9 ± 0.4	14.7 ± 4.1	15.0 ± 0.8	18.1 ± 0.5
	WB_10	7.8 ± 1.6	12.0 ± 0.1	11.8 ± 0.4	13.2 ± 0.3	14.8 ± 3.6	
	GC_10	8.0 ± 2.6	10.7 ± 2.6	16.9 ± 1.8	12.8 ± 1.4	21.3 ± 3.9	
	OB_5 + GC_5	6.9 ± 2.6	13.1 ± 1.5	12.9 ± 1.3	13.8 ± 0.2	18.7 ± 1.8	
	WB_5 + GC_5	9.2 ± 2.2	12.3 ± 0.5	16.3 ± 0.1	12.8 ± 0.6	15.6 ± 3.5	
	OB_20	4.2 ± 2.0	13.8 ± 0.1	18.7 ± 0.1	12.0 ± 0.3	18.4 ± 0.2	
	WB_20	7.9 ± 0.1	10.5 ± 2.2	13.2 ± 1.3	10.4 ± 0.9	13.7 ± 2.4	
	GC_20	8.4 ± 0.9	12.3 ± 0.1	14.7 ± 4.4	5.7 ± 1.9	14.4 ± 0.0	
	OB_10 + GC_10	11.5 ± 2.1	11.5 ± 1.4	18.5 ± 4.0	9.9 ± 3.4	15.9 ± 2.2	
	WB_10 + GC_10	8.4 ± 3.3	11.7 ± 2.7	17.1 ± 2.0	8.4 ± 1.4	17.4 ± 1.7	
	C/N _{at}	Control	57	38	36	54	28
		OB_10	38	35	37	35	34
WB_10		65	47	58	45	34	
GC_10		53	42	30	35	25	
OB_5 + GC_5		70	38	38	34	28	
WB_5 + GC_5		56	41	35	39	34	
OB_20		134	38	31	47	32	
WB_20		75	62	51	65	46	
GC_20		48	35	30	77	31	
OB_10 + GC_10		39	44	27	53	31	
WB_10 + GC_10		63	43	33	67	31	

¹ DAS: Days after sowing. ² *Medicago polymorpha*. ³ *Lolium perenne*. ⁴ TC: total carbon content. ⁵ TN: total nitrogen content. OB: olive pomace biochar; WB: wood biochar; GC: green compost. Different lowercase letters indicate significant differences ($p < 0.05$) between treatments for each type of pot. No letters indicate no significant differences.

No significant differences in TN were observed in the original substrate formulations. However, after 100 days, TN increased significantly, particularly in pots planted with *Medicago polymorpha*. This legume is known for its symbiotic nitrogen fixation through nodulation with *Rhizobium* spp., which enhances soil nitrogen availability [48,49].

The C/N ratio, an indicator of organic matter mineralization and nitrogen availability, declined over time, from a mean of 63.4 in the original mixes to 42.0 after incubation (Table 2). In planted substrates, mean C/N ratios were 36.9 for *M. polymorpha* and 50.1 for *L. perenne*. Treatments with 10–20% wood biochar maintained the highest C/N ratios, potentially leading to nitrogen immobilization and reduced uptake in *M. polymorpha* tissues (Table 3). These results underline the importance of balancing amendment dose and composition—not only for enhancing carbon content, but also for maintaining optimal nitrogen dynamics in peat-reduced growing media.

Table 3. Vegetation production and plant elemental composition (100 DAS—after harvest).

	Vegetation Development						Elemental Composition			
	Fresh Weight (g per Pot)	<i>M. polymorpha</i>		Fresh Weight (g per Pot)	<i>L. perenne</i>		<i>M. polymorpha</i>		<i>L. perenne</i>	
		Dry Weight (g per Pot)	Water Content (%)		Dry Weight (g per Pot)	Water Content (%)	TC (g kg ⁻¹)	TN (g kg ⁻¹)	TC (g kg ⁻¹)	TN (g kg ⁻¹)
Control	5.5 ± 1.6	2.7 ± 0.6	50.3 ± 4.3 ^{ab}	16.0 ± 2.5	0.6 ± 0.7	96.7 ± 3.8	428 ± 7 ^{bc}	30.4 ± 3.8 ^a	390 ± 4 ^b	46.3 ± 2.2 ^{abc}
OB_10	8.8 ± 3.2	3.1 ± 2.0	67.6 ± 13.8 ^a	13.0 ± 3.1	0.7 ± 0.4	95.2 ± 1.8	419 ± 5 ^c	19.7 ± 5.2 ^c	396 ± 2 ^b	49.6 ± 2.4 ^a
WB_10	6.0 ± 0.8	2.0 ± 0.5	65.2 ± 13.2 ^a	13.6 ± 0.5	0.7 ± 0.1	94.7 ± 0.5	426 ± 4 ^{bc}	20.7 ± 4.7 ^{bc}	408 ± 2 ^a	45.6 ± 0.1 ^{abc}
GC_10	7.5 ± 1.5	2.9 ± 0.5	61.2 ± 7.1 ^{ab}	12.0 ± 2.2	0.5 ± 0.3	95.7 ± 1.4	431 ± 7 ^{ab}	26.8 ± 3.9 ^{ab}	403 ± 1 ^a	47.6 ± 1.0 ^{ab}
OB_5 + GC_5	9.1 ± 3.5	2.5 ± 1.2	73.0 ± 3.8 ^a	14.7 ± 1.3	0.7 ± 0.5	95.6 ± 2.9	422 ± 1 ^{bc}	20.5 ± 0.4 ^{bc}	395 ± 1 ^b	49.8 ± 1.8 ^a
WB_5 + GC_5	6.5 ± 3.1	2.7 ± 0.8	56.0 ± 10.6 ^{ab}	13.0 ± 2.7	0.4 ± 0.4	97.1 ± 2.3	428 ± 0 ^{bc}	20.9 ± 2.2 ^{bc}	409 ± 2 ^a	42.3 ± 2.5 ^{bcd}
OB_20	-	-	-	-	-	-	-	-	-	-
WB_20	6.7 ± 0.3	2.7 ± 0.2	58.8 ± 2.3 ^{ab}	13.3 ± 1.3	0.7 ± 0.2	95.1 ± 0.8	440 ± 5 ^a	24.0 ± 0.1 ^{abc}	406 ± 4 ^a	46.6 ± 1.1 ^{abc}
GC_20	5.3 ± 2.1	1.7 ± 0.6	68.2 ± 2.1 ^a	13.0 ± 1.9	0.7 ± 0.3	94.8 ± 1.5	424 ± 6 ^{bc}	25.3 ± 0.7 ^{abc}	403 ± 3 ^a	44.9 ± 0.9 ^{abc}
OB_10 + GC_10	8.2 ± 5.4	3.0 ± 2.3	69.2 ± 12.7 ^a	14.1 ± 3.2	0.6 ± 0.7	96.5 ± 4.0	429 ± 3 ^{abc}	21.7 ± 1.1 ^{bc}	403 ± 3 ^a	40.3 ± 2.3 ^{cd}
WB_10 + GC_10	5.5 ± 0.2	3.1 ± 0.1	43.4 ± 2.0 ^b	14.1 ± 1.7	0.9 ± 0.3	94.0 ± 1.3	431 ± 4 ^{ab}	21.9 ± 0.9 ^{bc}	406 ± 4 ^a	37.8 ± 0.4 ^d

DAS: Days after sowing. TC: total carbon content. TN: total nitrogen content. OB: olive pomace biochar; WB: wood biochar; GC: green compost. Different lowercase letters indicate significant differences ($p < 0.05$) between treatments for each type of plant. No letters indicate no significant differences.

Figure 4 illustrates the substrates' moisture measured weekly in control pots without plants, pots planted with *L. perenne*, and pots planted with *M. polymorpha* from experiment I. Substrates' moisture were comparable among all treatments, although control tended to be low. Moisture of substrate with 20% of OB and OB_10 + GC_10 was notable in pots where *M. polymorpha* was planted. The effect can plausibly be explained by the poor growth of this plant with OB at 20%. Despite occasional drops in pot humidity due to minor fluctuations in greenhouse conditions, the study's amended treatments generally showed resilience. In contrast, the control treatment was observably affected by these same events. Additionally, total moisture was measured at 100 DAS in Experiment II (Figure 5), humidity being comparable in all substrates. Though biochar has been widely demonstrated to increase soil moisture due to its high WHC [7], the commercial substrate used in this experiment already had very high WHC. The amendments showed varying effectiveness as peat substitutes; however, none influenced the stem water content of olive saplings. Other authors reported reductions in water retention when rice husk or tomato plant biochars were used in peat-based growing media [12]. As expected, pots planted with olive saplings consistently showed lower total moisture (red bars) compared to the unplanted control pots (blue bars), confirming water consumption by the plants. Additionally, a ratio representing water loss related to evapotranspiration was calculated between pots with olive saplings and pots without plants (Figure 5). This colored-scale ratio provides further insight into the issue: a positive proportion indicates higher water loss from the planted pots. This ratio was particularly high for the WB_5 + GC_5 treatment (0.45), suggesting significant water consumption by the plant. However, a negative ratio was observed for the OB_10 treatment (−0.20); pots where olives did not grow or survived. Positive Pearson correlations ($p < 0.05$)

were found between this ratio and the fresh weight of leaves and also with the fresh weight of stems.

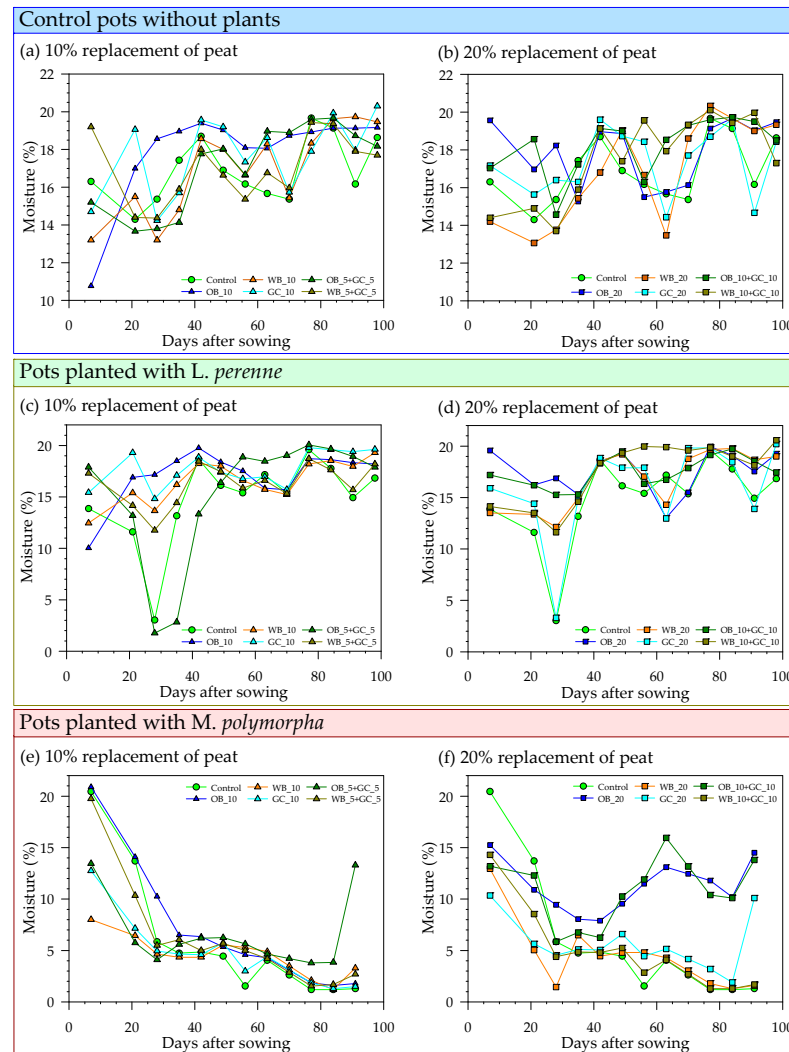


Figure 4. Substrate moisture (%) in Control pots without plants (a,b), pots planted with *L. perenne* (c,d), and pots planted with *M. polymorpha* (e,f): Control and treatments with 10% of peat substitution are represented in (a,c,e); Control and treatments with 20% of peat substitution are represented in (b,d,f). Green circle symbol denotes the control (commercial peat substrate). Triangles represent treatments with 10% substitution of peat, and squares represent treatments with 20% peat substitution.

3.3. Plant Yield, Physiological Performance and Nutrient Uptake Under Peat-Reduced Substrates

3.3.1. Experiment I: Biomass and Carbon Uptake in Forage Species

Substrate substitution had no significant effect on the fresh and dry biomass or water content (%) of *Medicago polymorpha* and *Lolium perenne* after 100 days (Table 3), suggesting the proposed amendments are agronomically viable for partial peat replacement. The only exception was the 20% OB treatment, in which seedling establishment failed, preventing any further measurements. Gascó et al. [34] showed increments in biomass production of *L. perenne* when peat was replaced by biochar from the organic fraction of municipal solid waste, but no significant effect when it was replaced by biochar from pinewood.

Total carbon content in *L. perenne* tissues increased with the addition of wood biochar and green compost, with the exception of treatments containing OB alone, which did not enhance plant carbon uptake. These findings support the potential of compost and wood-derived biochar to maintain plant productivity while improving substrate carbon stocks. However, OB at high doses may negatively impact early plant development, likely

due to its elevated salinity or nutrient imbalance [7]. This is in accordance with Nocentini et al. [12], who reported reduced plant growth when peat was replaced with biochars produced from wood-poor green wastes due to their high salt concentration.

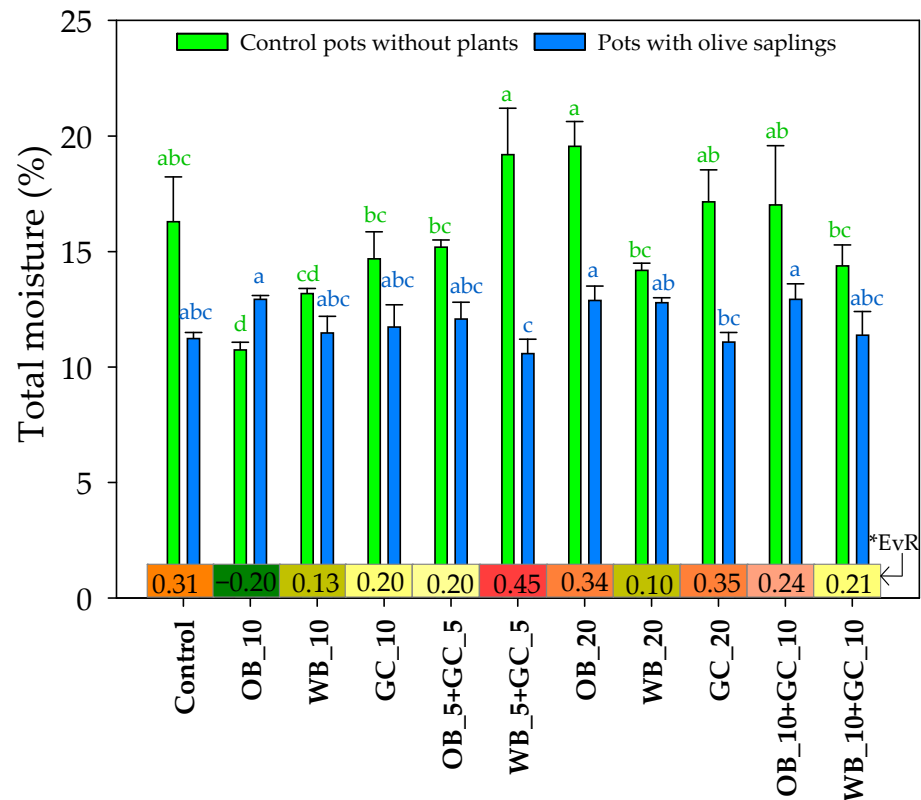


Figure 5. Total moisture (%) in pots without plants and in pots planted with olive saplings (100 DAS). Different green lowercase letters indicate significant differences between treatments in pots without plants ($p < 0.05$). Different blue lowercase letters indicate significant differences between treatments in pots with olive saplings ($p < 0.05$). *EvR: the evapotranspiration ratio is represented in the colored scale over the bars [The scale ranges from lower rates (green) to higher rates (red)]. This ratio was determined from the volumetric moisture loss in pots with olive saplings compared to control pots without plants for each treatment.

3.3.2. Experiment II: Olive Trees Performance and Nutrient Status

In *Olea Europaea* var. *Arbequina* saplings, no significant differences in leaf and stem biomass were observed between the control and treatments with WB or GC alone (Table 4). Nevertheless, treatments containing OB, particularly at 20%, showed reduced biomass accumulation. Tosca et al. [50] did not find differences in olive height when biochar and compost were used for peat replacement but did report reduced branching when biochar was used at 20%. Photosynthetic parameters, including SPAD and quantum yield, are presented in Table 4 for the middle section of the olive saplings. While measurements were also taken in the upper section (as detailed in Section 2.3.3 Pot experiment II), those data showed no significant differences and are therefore not shown. Chlorophyll status was assessed through SPAD index measurements, taken before and after irrigation to exclude water stress as a confounding factor [51]. No significant differences were found between time points or treatments, confirming the absence of irrigation-related stress (Table 4). The SPAD index is widely used as a proxy for chlorophyll and nitrogen content in leaves [52,53], and its stability here suggests no evident nutrient deficiency symptoms. This is crucial in olive cultivation, where both nitrogen deficiency and excess can affect yield and oil quality [54,55]. Quantum yield (QYPSII) values, a marker of photosynthetic efficiency

and plant stress [11], did not differ significantly among treatments, further confirming physiological stability across the amended substrates.

Table 4. Vegetation development and water and stress-indicating parameters of olive plants cultivated in different substrates (100 DAS).

	Plant Height (cm)	Fresh Weight of Leaves (g)	Dry Weight of Leaves (g)	Water Content in Leaves (%)	Fresh Weight of Stems (g)	Dry Weight of Stems (g)	SPAD Index [Ms]		Quantum Yield ($F' m F' v^{-1}$) [Ms]	
							Before Irrigation	After Irrigation	Before Irrigation	After Irrigation
Control	59.4 ± 8.8 ^{ab}	14.6 ± 4.6 ^a	6.7 ± 2.1 ^{ab}	54.3 ± 2.1 ^b	7.2 ± 1.4 ^a	3.8 ± 0.7 ^a	79 ± 4	83 ± 3	0.73 ± 0.02	0.73 ± 0.03 ^a
OB_10	10.7 ± 2.7 ^c	0.5 ± 0.4 ^b	0.2 ± 0.1 ^d	62.1 ± 0.1 ^a	0.5 ± 0.3 ^d	0.3 ± 0.1 ^c	68 ± 24	74 ± 19	0.69 ± 0.06	0.67 ± 0.07 ^{ab}
WB_10	58.8 ± 7.6 ^{ab}	14.6 ± 1.6 ^a	6.4 ± 0.8 ^{ab}	56.5 ± 0.8 ^{ab}	6.7 ± 2.3 ^{ab}	3.3 ± 1.2 ^a	75 ± 5	77 ± 6	0.68 ± 0.09	0.68 ± 0.07 ^{ab}
GC_10	61.2 ± 4.1 ^a	14.7 ± 1.5 ^a	6.5 ± 0.8 ^{ab}	55.8 ± 0.8 ^{ab}	6.9 ± 0.9 ^{ab}	3.5 ± 0.5 ^a	80 ± 4	82 ± 5	0.73 ± 0.05	0.70 ± 0.03 ^{ab}
OB_5 + GC_5	41.9 ± 25.0 ^{ab}	7.4 ± 5.1 ^{ab}	3.0 ± 1.9 ^c	56.5 ± 2.0 ^{ab}	4.3 ± 3.1 ^c	1.9 ± 1.4 ^b	67 ± 19	73 ± 17	0.62 ± 0.09	0.59 ± 0.11 ^b
WB_5 + GC_5	54.9 ± 10.9 ^{ab}	15.2 ± 3.5 ^a	6.8 ± 1.5 ^a	55.5 ± 1.5 ^{ab}	6.2 ± 1.2 ^{ab}	3.1 ± 0.8 ^a	79 ± 5	76 ± 7	0.68 ± 0.08	0.62 ± 0.07 ^{ab}
OB_20	7.6 ± 0.5 ^d	0.0 ± 0.0 ^b	0.0 ± 0.0 ^d	-	0.2 ± 0.0 ^d	0.1 ± 0.0 ^c	-	-	-	-
WB_20	55.5 ± 4.6 ^b	10.3 ± 3.3 ^a	4.7 ± 1.0 ^{abc}	51.5 ± 1.0 ^{ab}	5.5 ± 0.9 ^{bc}	2.7 ± 0.4 ^{ab}	71 ± 4	73 ± 5	0.73 ± 0.01	0.73 ± 0.02 ^a
GC_20	56.2 ± 5.6 ^{ab}	10.3 ± 5.0 ^a	4.6 ± 2.3 ^{bc}	54.9 ± 2.3 ^{ab}	5.8 ± 1.4 ^{abc}	2.9 ± 0.8 ^{ab}	80 ± 6	81 ± 3	0.71 ± 0.08	0.68 ± 0.09 ^{ab}
OB_10 + GC_10	8.8 ± 2.7 ^{cd}	0.2 ± 0.2 ^b	0.1 ± 0.1 ^d	44.5 ± 0.1 ^b	0.4 ± 0.2 ^d	0.2 ± 0.1 ^c	89 ± 3*	89 ± 4*	0.74 ± 0.02*	-
WB_10 + GC_10	54.0 ± 4.1 ^b	14.0 ± 3.3 ^a	6.3 ± 1.5 ^{ab}	55.2 ± 1.5 ^{ab}	6.4 ± 1.5 ^{ab}	3.3 ± 0.9 ^a	79 ± 2	79 ± 4	0.64 ± 0.07	0.63 ± 0.10 ^{ab}

DAS: Days after sowing. Ms: middle section. OB: olive pomace biochar; WB: wood biochar; GC: green compost. *: Only two pots contained one plant where analysis could be performed. Different lowercase letters indicate significant differences between treatments ($p < 0.05$). No letters indicate no significant differences.

3.3.3. Nutrient Uptake and Elemental Composition of Olive Plants

All the elements shown in Table 5 excepting C are essential nutrients required for key physiological and metabolic processes in plants or contribute to optimal growth and vigor [56]. Elemental analyses of olive shoot tissues revealed no major differences in macronutrient and micronutrient concentrations between the control and amended treatments, except for magnesium (Mg) and potassium (K) (Table 5). Notably, the OB_5 + GC_5 treatment exhibited significantly higher K concentrations compared to the control, likely reflecting the K-rich nature of the OB amendment (47.88 mg g⁻¹ vs. 6.11 mg g⁻¹ in GC), a fact which agrees with findings by Fornes et al. [57], who reported altered K and Ca uptake in olive leaves following compost or biochar applications. Although Mg concentrations were generally low, they remained within the range reported for young olive trees not yet in fruiting stage [58]. Importantly, all nutrient concentrations—including total N—were comparable to those in control plants and exceeded the minimum sufficiency levels defined by Fernández-Escobar [59], with Mg as the only exception. As no signs of deficiency, stress, or biomass loss were detected in any treatment, the absence of significant differences in olive plant nitrogen content provides a key rationale for using these amendments as peat substitutes. This is particularly relevant given the direct relationship between nitrogen and protein content. When estimating the latter, a conversion factor of 6.25 is typically applied, assuming a 16% nitrogen content in proteins, conceding that this is an estimated and generalized value [60]. These findings confirm that peat-reduced substrates can support normal nutrient uptake and physiological functioning in olive saplings, even over relatively short trial durations.

All nutrient concentrations, with the exception of Mg, exceeded deficiency thresholds established by Fernández-Escobar [59]. Importantly, despite lower Mg levels, no visual deficiency symptoms or reductions in physiological performance were observed during the experiment. Replacing peat partially with biochar and/or GC maintains optimal nutrient assimilation and physiological integrity in olive saplings, demonstrating no compromise in plant quality in short-term trials.

Table 5. Total content of macronutrients and micronutrients, and elemental composition of olive trees at the end of the experiment (100 DAS).

	Nutrients										Elemental Composition		
	Ca (mg g ⁻¹ DW)	K (mg g ⁻¹ DW)	Mg (mg g ⁻¹ DW)	Na (mg g ⁻¹ DW)	P (mg g ⁻¹ DW)	S (mg g ⁻¹ DW)	B (μg g ⁻¹ DW)	Cu (μg g ⁻¹ DW)	Fe (μg g ⁻¹ DW)	Mn (μg g ⁻¹ DW)	Zn (μg g ⁻¹ DW)	TC (g kg ⁻¹)	TN (g kg ⁻¹)
Control	3.68 ± 0.10 ^{ab}	12.8 ± 0.62 ^b	0.94 ± 0.11 ^a	0.06 ± 0.04	1.52 ± 0.26	1.80 ± 0.22 ^{ab}	17.20 ± 1.79	0.82 ± 0.18	43.42 ± 8.30	21.26 ± 3.43 ^{ab}	6.51 ± 3.4 ^{ab}	480 ± 10	19.0 ± 1.9 ^{ab}
OB_10*	7.84	18.23	0.81	0.41	2.19	2.24	16.68	3.04	48.10	22.84	8.48	442	23.8
WB_10	3.95 ± 0.26 ^{ab}	14.0 ± 1.60 ^{ab}	0.65 ± 0.04 ^b	0.12 ± 0.07	1.22 ± 0.19	1.60 ± 0.22 ^{ab}	16.90 ± 2.32	0.85 ± 0.17	36.86 ± 4.31	15.47 ± 0.56 ^b	5.46 ± 0.5 ^{ab}	467 ± 47	15.9 ± 3.2 ^{ab}
GC_10	3.69 ± 0.24 ^{ab}	12.6 ± 0.39 ^b	0.55 ± 0.03 ^b	0.07 ± 0.05	1.34 ± 0.15	1.69 ± 0.33 ^{ab}	17.40 ± 2.28	1.58 ± 1.24	35.50 ± 4.62	13.31 ± 1.52 ^b	5.70 ± 1.5 ^{ab}	474 ± 3	17.8 ± 1.8 ^{ab}
OB_5+	3.13 ±	19.2 ±	0.52 ±	0.38 ±	1.57 ±	1.95 ±	19.15 ±	0.87 ±	44.86 ±	21.03 ±	7.09 ±	472 ±	23.1 ±
GC_5	0.13 ^b	2.53 ^a	0.06 ^b	0.19	0.27	0.26 ^a	2.49	0.07	6.90	1.30 ^a	1.3 ^a	15	3.5 ^a
WB_5+	4.33 ±	14.4 ±	0.70 ±	0.17 ±	1.51 ±	1.61 ±	21.88 ±	0.76 ±	37.33 ±	19.45 ±	6.06 ±	475 ±	16.7 ±
GC_5	0.59 ^a	1.68 ^{ab}	0.09 ^b	0.14	0.14	0.17 ^{ab}	3.50	0.10	6.22	9.51 ^{ab}	9.5 ^{ab}	26	1.5 ^{ab}
OB_20**	-	-	-	-	-	-	-	-	-	-	-	-	-
WB_20	3.81 ± 0.47 ^{ab}	16.3 ± 0.93 ^{ab}	0.64 ± 0.09 ^b	0.10 ± 0.09	1.31 ± 0.17	1.41 ± 0.20 ^b	19.52 ± 1.80	1.88 ± 1.91	33.81 ± 3.74	17.30 ± 1.51 ^{ab}	9.51 ± 1.5 ^{ab}	470 ± 8	13.9 ± 2.5 ^b
GC_20	3.83 ± 0.22 ^{ab}	16.0 ± 0.46 ^{ab}	0.56 ± 0.10 ^b	0.11 ± 0.07	1.44 ± 0.09	2.01 ± 0.19 ^a	19.72 ± 0.75	2.75 ± 1.18	39.02 ± 2.87	16.39 ± 0.52 ^b	9.80 ± 0.5 ^{ab}	469 ± 19	20.0 ± 3.3 ^{ab}
OB_10+ GC_10**	-	-	-	-	-	-	-	-	-	-	-	-	-
WB_10 +GC_10	3.99 ± 0.10 ^{ab}	14.1 ± 1.08 ^{ab}	0.55 ± 0.06 ^b	0.08 ± 0.09	1.42 ± 0.27	1.70 ± 0.35 ^{ab}	19.76 ± 2.67	0.75 ± 0.18	36.47 ± 5.20	15.14 ± 4.03 ^b	5.36 ± 4.0 ^b	458 ± 15	18.0 ± 1.6 ^{ab}

DAS: Days after sowing. DW: Dry weight of olive plants. TC: total carbon content. TN: total nitrogen content. OB: olive pomace biochar; WB: wood biochar; GC: green compost. * This treatment did not have enough plant material for performing replicates. ** This treatment did not have enough plant material for analysis. Different lowercase letters indicate significant differences between treatments ($p < 0.05$). No letters indicate no significant differences.

4. Conclusions

This study demonstrates that the partial replacement of peat with biochar and/or green compost as formulated above will significantly improve the physicochemical properties of horticultural substrates, particularly by increasing total carbon content and buffering substrate pH. Although substantial increases in plant productivity were not observed, neither biomass accumulation nor physiological performance were negatively affected in forage species or olive saplings, even during the early and more sensitive growth stages. The experiment's outcome indicates that these materials preserve agronomic functionality while reducing peat dependence—a crucial step toward more sustainable nursery and substrate management.

Once proven that the success of biochar application relies on its physicochemical properties, the responses between the two tested biochars point to the relevance of proper feedstock selection and pyrolysis conditions. The limited performance of olive pomace biochar at higher application rates (20%) underscores the need for proper characterization and dosage optimization before use in plant production. In contrast, wood biochar and green compost prove to be reliable alternatives capable of improving carbon content without impairing germination or plant development. The current economic cost of biochar may be offset both by its contribution to long-term carbon sequestration, and by the potential to generate syngas useful as biofuel and other products during pyrolysis, especially when produced locally from residual feedstock. As restrictions on peat use intensify, biochar ought to become a feasible and sustainable option. Moreover, when properly selected, characterized, and applied, biochar and compost can effectively replace part of peat in growing media. Their use supports nutrient balance and carbon sustainability, while advancing the transition toward peat-free and low-impact horticulture, fostering circular and environmentally responsible practices in the field.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy15112455/s1>: Figure S1. Study design diagram.

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Abbreviations

The following abbreviations are used in this manuscript:

LP	<i>Lolium perenne</i>
MP	<i>Medicago polymorpha</i>
LR	<i>Lolium rigidum</i>
FA	<i>Festuca arundinacea</i>
EC	Electrical Conductivity
SPAD	Soil–Plant Analysis Development
Ms	Middle section
WHC	Water holding capacity
OB	Olive pomace Biochar
WB	Wood Biochar
GC	Green Compost
DAS	Days After Sowing

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