



# *Article* **Sediment-Based Growing Media Provides a Window Opportunity for Environmentally Friendly Production of Ornamental Shrubs**

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**Abstract:** Sediments remediated with a nature-based solution approach (NBS-sediments) can represent a suitable and affordable alternative to peat as a constituent of growing media for ornamental plant production based on the combination of advanced production efficiency and rational green use of resources, including peat and water. In a greenhouse experiment, the effect of different growing media containing NBS-remediated sediments on two-year-old container grown cherry laurel (*Prunus laurocerasus* cv. 'Novìta') under standard and induced restrictive irrigation was evaluated. Six ternary mixes with different proportion (45:30:25 and 30:20:50 *v*/*v*) of peat:pumice:sediment (PE:TS25, PE:TS50), coconut fiber:pumice:sediment (CF:TS25, CF:TS50) and wood fiber:pumice:sediment (WF:TS25, WF:TS50) were tested in comparison to the standard peat:pumice blend (60:40 *v*/*v*), commonly used for pot ornamental crops ad used as control (PE, control). Pots were drip irrigated with 200 and 250 cc daily water volume (DWV). Cherry laurels grown in the control showed the lowest sign of stress, maintaining the highest net CO<sub>2</sub> assimilation and transpiration rates, however stomatal conductance was reduced compared to PE:TS mixes. On the other hand, photosynthetic performance was strongly depressed by WF:TS25 and WF:TS50 under reduced DWV compared to the control, due to the combined effect of physical properties of the used matrices and reduced water availability. Nevertheless, final biomass production of plants grown on sediment-based growing media was similar to that of control, indicating that photosynthetic performance of plants fully recovered during the cultivation period. Differences in final plant development were negligible when compared to quality standards of marketing categories. Thus, appropriately blended NBS-sediment-based growing media can be used on a larger scale to produce rustic outdoor ornamentals.

**Keywords:** *Prunus laurocerasus*; nursery industry; sustainability; peat; coconut fiber; wood fiber; water management; plant development; plant physiology

# **1. Introduction**

Europe is traditionally the largest producer of flower and ornamental plants in the world with a net exportation of pot plants, conifers, hardy perennials, bulbs, and corms. The Netherlands, Italy, Germany, Spain, United Kingdom and France are the main production centers [\[1\]](#page-16-0).

In Italy the ornamental plant industry is concentrated mainly in Tuscany, especially in the province of Pistoia, which represents the heartbeat of the Italian nursery stock production. Thanks to its unique microclimate, the Pistoia nursery industry produces all year-long a huge assortment, from evergreen and deciduous subshrubs to big trees, conifers, roses, palms, acidophilic plants, water plants hedges, vines, fruit trees and lawns of high quality, representing a point of reference at both national and European level. Currently,



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the Tuscan ornamental plant sector specialized in outdoor plants covers approximately a quarter of the Italian ornamental production, encompassing more than 5000 ha of which over 900 in containers, thanks to the activity of over 1400 nursery enterprises, and is recognized as one of the most important leaders in Europe for outdoor ornamental plants. This sector is largely based on evergreen shrubs and trees  $(\sim 31\%)$ . The production facilities of plant nurseries are highly organized. Plants are grown in containers or in the open field. Plants are often propagated by seed or by cuttings in pots where they remain until the sale, otherwise they are transplanted into the ground or transferred in larger containers [\[2–](#page-16-1)[5\]](#page-16-2).

The production of ornamental plants is characterized by highly intensive processes and the use of resources, having a high impact on the environment [\[6\]](#page-16-3). In particular, the contribution to  $CO<sub>2</sub>$  in container production nurseries depends mainly on two specific factors: the peat component used in the preparation of growing media and the considerable amount of plastic, essentially pots and irrigation pipes [\[3\]](#page-16-4). Hence, the modern production of ornamental plants requires solutions that combine advanced production efficiency with a more rational and environmentally friendly use of resources, including peat and water. One of the elements of sustainable development in ornamental crop production is technological progress achieved by implementing the use of sustainable substitutes as potted substrate.

European funding and commercial policies support and encourage the use of sustainable peat alternatives that must meet specific technical requirements depending on the nursery production sector. Recently, several studies aimed to find adequate peat alternatives have proposed different organic substrates as partial peat substitute for ornamental crop production [\[7\]](#page-16-5). Moreover, the recent and growing interest in waste recycling has led to an increased use of organic waste as soil amendment or substrate component [\[8\]](#page-16-6).

Among the waste materials, the phytoremediated marine sediment is a relatively inert matrix with a lower carbon footprint that can be incorporated to reduce peat content in growing media recipes, representing at the same time a smart solution to the problem of waste disposal. More recently, the research community moved further towards the adoption of organic wetting agent used for reducing the risks of peat hydrophobicity occurring during its drying [\[5,](#page-16-2)[9\]](#page-16-7). In this context, the bioremediated sediment could represent a valid means to increase the water capacity of growing media mixes while reducing the peat fraction.

Little is currently known on the compatibility and combinability of the phytoremediated sediment with other standard matrixes  $[10-17]$  $[10-17]$ . So, it is yet not possible to define a mix that would be certain to provide good properties, without extensive case by case testing. This work aimed to explore windows of opportunity for sustainable production of commercially important ornamental plant species on sediment enriched substrates. Several studies have shown that water amount and substrate type are key factors influencing plant growth [\[18\]](#page-17-1); thus, the influence of substrate characteristics and irrigation interaction on water and nutrient uptake by plants was investigated. *Prunus laurocerasus* cv. 'Novita' was chosen as representative evergreen Tuscan ornamental species. Known as cherry laurel as well, it is particularly hardy and vigorous species, widely used as barrier plant (hedge), thanks to its large, and glossy foliage, spreading habit and very fast growing and plant development. The objective of the present work was to study the properties of bioremediated sediment mixed in different proportion with organic substrates and its influence on 2-year-old *P. laurocerasus*, obtained by rooted cuttings according to Tozzi et al. [\[19\]](#page-17-2) and typically marketed in containers of 8.5 L.

## **2. Materials and Methods**

## *2.1. Plant Material and Experimental Conditions*

A greenhouse experiment was conducted to test the effect of different growing media containing a bioremediated sediment fraction and an induced restrictive irrigation on the growth of cherry laurel (*P. laurocerasus* L.) cv. 'Novìta'. This species tolerates most soil types and is surprisingly hardy, thus it can withstand the harsh winter temperatures and is adapted to both mild and continental climate type. It quickly forms a dense and substantial

crown that will block the window views from surrounding houses, or provide an excellent evergreen barrier for the front yard. The cultivar 'Novìta', selected in Holland, has now become widely established and has replaced the previous cultivar 'Caucasica', compared to which it has longer, thicker, and shinier leaves, and above all, is more resistant to diseases and water stress.

The study was carried out in a polyethylene-covered  $132 \text{ m}^2$  greenhouse located in Montecarlo (PT) (43°51′31.9″ N, 10°41′11.4″ E, Tuscany, Italy). Pot experiments were carried out on five raw materials, whose main characteristics are reported in Table [1:](#page-2-0) (i) a treated sediment (TS), 1.19 g cm<sup>-3</sup> bulk density, dredged from the Leghorn Port (Tuscany,  $43°33'25''$  N,  $10°17'39''$  E); (ii) a white milled peat (PE), 0.11 g cm<sup>-3</sup> bulk density, extracted in Lithuania; (iii) a coconut fiber (CF) 0.11 g cm<sup>-3</sup> bulk density, of Indian (Tamil Nadu) origin; (iv) a wood fiber (WF), 0.08 g cm<sup>-3</sup> bulk density, purchased from Fibra di Legno s.r.l. (Como, Italy); (v) a pumice (Pu), 0.51 g cm<sup>-3</sup> bulk density, from Lipari (IT) volcano deposit. The treated sediment results from a process of phytoremediation and landfarming as described by Tozzi et al. [\[15\]](#page-16-9).



<span id="page-2-0"></span>**Table 1.** Main characteristics of raw materials used for growing media preparation.

Electrical conductivity. Total nitrogen (TN). Total organic carbon (TOC). Total phosphorus (TP). Limit of detection (0.01 ppm) (LOD).

Six ternary mixes with different proportion (45:30:25 and 30:20:50 *v*/*v*) of PE:Pu:TS (PE:TS25, PE:TS50), CF:Pu:TS (CF:TS25, CF:TS50) and WF:Pu:TS (WF:TS25, WF:TS50) were tested in comparison to the standard peat:pumice blend (60:40 *v*/*v*), commonly used for pot ornamental crop production in Tuscany, which was considered as the control (PE) (Table [2\)](#page-3-0).



<span id="page-3-0"></span>**Table 2.** Composition of the tested growing media.

Due to its dominant national and international position within the ornamental plant sector, *Prunus laurocerasus* cv. 'Novita' was chosen as representative evergreen outdoor ornamental species. One-year-old rooted *P. laurocerasus* plants from a previous trial on cutting propagation were used (Figure S1) [\[19\]](#page-17-2). Plants (average height = 50.1 cm) were obtained without any hormone application and were grown without the use of chemical pesticide. The rooted cuttings were grown in 8.5 L drip-irrigated pots. To better understand the effect of water retention caused by the incorporation of the sediment into the substrate, plants were supplied with a daily water volume (DWV) of 200 cc and 250 cc per pot. Delivered water amounts were chosen based on the most efficient nursery industry water management applied in Tuscany for this species with less over-watering and minimum water runoff. Recirculating drip irrigation was used to control water supply and all plants were irrigated from the same reservoir. Two different line drippers were used to control water supply with one drip emitter per pot at a flow rate of 80 cc min<sup>-1</sup> and irrigation timing varying from 2.5 to 3.2 min per day. The pH of irrigation water was maintained between 6.0 and 6.5.

A randomized complete block design was used with seven growing media (GM = PE, as control; PE:TS25; PE:TS50; CF:TS25; CF:TS50; WF:TS25; WF:TS50) and two irrigation volumes (DWV 200, DWV 250). The growth trial started in April 2021 with three replications of each treatment, and three cherry laurel plants in each replication. Thus, a total of fourteen treatments were evaluated, with nine cherry laurel plants grown per treatment (*n* = 9). All pots were fertigated weekly (160 cc/pot) with a soluble fertilizer (Universol 15-7-30, ICL Italy s.r.l., Milano, Italy) dosed at 10.5  $\rm g L^{-1}$ . The trial was finished in March 2022 with the physical and chemical characterization of the growing media and the destructive analyses of the plant material.

## *2.2. Physical, Chemical and Biochemical Properties of Growing Media*

Three representative composite samples of each growing media were tested for physical properties and chemical composition before and after the cultivation experiment. Methods for characterization of the considered mixes were based on European Standards developed by ECN. Bulk density (BD), particle density (PD), total pore space (TPS), water content (WC), air content (AC) and available water (AW) were evaluated with UNI EN 13,041 [\[20\]](#page-17-3) protocol with sandbox for pF determination (Royal Eijkelcamp, Giesbeek, The Netherlands). WC represented the water volume content expressed as a percentage by volume at water pressure of  $-1$  kPa ( $-10$  cm); AC was the air volumetric content expressed as a percentage by volume, in %  $(v/v)$ , with  $-1$  kPa as pressure head; AW, the available water for plant growth, was calculated as the difference between the water volume content expressed as a percentage by volume at water pressure of −1 kPa (−10 cm) and −10 kPa (-100 cm). To measure pH, electrical conductivity (EC), ammonia (N-NH<sub>4</sub><sup>+</sup>) and nitrates (N-NO<sup>3</sup> <sup>−</sup>) of the growing media, an aqueous suspension was prepared in a 1:5 (*v*/*v*) ratio of growing media to water, shaken at 250 rpm for 1 h (UNI EN 13,038 [\[21\]](#page-17-4) and UNI EN

13,037 [\[22\]](#page-17-5)). The determinations were performed using selective electrodes as follows: Conmet 2, (Hanna Instruments, Woonsocket, Rhode Island, USA) for EC; GSE ammonia electrode (Sevenmulti, Mettler Toledo, Greifensee, Switzerland for N-NH<sub>4</sub><sup>+</sup>; DX262-NO<sub>3</sub> ISE half-cell electrode (Sevenmulti, Mettler Toledo, Greifensee, Switzerland) for N-NO<sub>3</sub><sup>-</sup>; pH electrode InLab routine pro (Sevenmulti, Mettler Toledo, Greifensee, Switzerland) for pH; each measurement was carried out according to each manufacture's instructions.

Total P (TP) were determined using colorimetric analysis (Spectophotometer-Unicam UV 500; Thermo Fisher Scientific, Waltham, MA, USA) [\[23\]](#page-17-6) after acid digestion with hydrogen peroxide-nitric acid ( $H_2O_2/HNO_3$  1:3). Total organic C (TOC) and total N content (TN) were determined by dry combustion with FlashSmart Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Ca, Mg, Na, K, Fe, Mn, Cu, Zn, Ni, Cr, Pb, Cd were analyzed by ICP (5900 ICP-OES; Agilent, Santa Clara, CA, USA) after acid digestion in microwave (Ethos 1; Milestone srl, Bergamo, Italy) with hydrogen peroxidenitric acid H2O2/HNO<sup>3</sup> 1:3 *v*/*v*. β-1.4-glucosidase (β-Glu), acid phosphatase (Acid-P), butyrate esterase (B-Est) and arylsulfatase (Aryl-S) were measured on Infinite F200 pro plate reader fluorimeter (Tecan, Männedorf, Zürich, Switzerland) according to Marx et al. [\[24\]](#page-17-7) and Vepsäläinen et al. [\[25\]](#page-17-8) protocols based on the use of fluorogenic methyl-umbelliferyl (MUF) substrates.

# *2.3. Plant Growth and Biomass Production*

Base stem diameter (BSD), maximum plant height (MPH), number of vegetative sprouts (NVS), length of vegetative sprouts (LVS), and number of fully expanded leaves on vegetative sprouts (NEL) were recorded on June, September, December 2021. For each assessment, the cumulative number of developed shoots and leaves were considered, while LVS was measured intermittently only on the new sprouts.

At the end of the trial, plant stem dry weight (SDW), leaf dry weight (LDW), root dry weight (RDW), and total leaf area (TLA) were determined. To obtain dry plant areal and root weights, samples were dried in a forced-air oven at 75 ◦C to a constant weight and then weighted. The total leaf area per plant was measured using an electronic leaf area meter (WinDIAS Image Analysis System, Delta-T Devices, UK). The relative growth rate (g  $g^{-1}$  day<sup>-1</sup>) was calculated using the equation reported by De Groot et al. [\[26\]](#page-17-9):  $RGR = (lnW2 - lnW1)/(t2 - t1)$  where W1 and W2 are the fresh masses (g) of the aboveground plant part at times t1 and t2, corresponding to the beginning (1 DAT) and to the end of the experiment (346 DAT), respectively.

## *2.4. Physiological Analyses*

Measurements of leaf gas exchanges were conducted during full vegetative activity at 75 DAT within 2 h across solar noon (i.e., between 11:00 and 13:00 h) on the youngest fully expanded leaves, using six replicates per each treatment. The net  $CO<sub>2</sub>$  assimilation rate (Pn), stomatal conductance (Gs), transpiration rate (E), water use efficiency of photosynthesis (WUEPn), and sub-stomatal  $CO<sub>2</sub>$  concentration (Ci) were determined with a portable gas exchange analyzer (Portable Photosynthesis System Ciras-2, PPSsystems, Amesbury, MA 01913 USA), equipped with a broad-leaf PLC (cuvette window area, 6.25 cm<sup>2</sup>). PAR, R.H., and carbon dioxide concentrations were set at ambient value and the flow rate of air was  $400 \text{ mL s}^{-1}.$ 

Colorimetric analyses were performed on leaf samples collected immediately after evaluation of photosynthetic performance (75 DAT) and at the end of plant vegetative growth (346 DAT). Chroma index was calculated using the coordinates  $a^*$  and  $b^*$ (Chroma =  $[(a^*2 + b^*2)^{1/2}]$ , where a<sup>\*</sup> [redness], and b<sup>\*</sup> [yellowness], were measured with a Chromameter, Minolta CR 200 colorimeter (XRITE, SP64, MI, USA) on three different blade points.

Pigment analysis (chlorophylls and carotenoids) were performed shortly after photosynthesis measurements. Five fully expanded leaves from each GM x DWV plot were selected from the middle portion of the plants for leaf disc preparation, and the methanolic

extraction technique of Lichtentahler and Buschmann [\[27\]](#page-17-10) was used for chlorophylls extraction prior to spectrophotometric absorbance reading (Thermo Evolution 300 UV-Visible Spectrophotometer).

During the same period three liquid nitrogen frozen samples of each GM x DWV plot were used for lipid peroxidation assessment. MDA levels were estimated according to the corrected TCA and TBA method as described by Wang et al. [\[28\]](#page-17-11). Ion's analysis was also performed on homogenized leaf samples in triplicates for each treatment. The leaf dry tissues were finely ground, and element concentration was determined by ICP after mineralization with microwave-assisted acid digestion (Ethos 1, Milestone) (ECN 2007).

# *2.5. Statistical Analysis*

All collected data were subjected to a two-way analysis of variance (ANOVA) to determine treatment effects. Where significant effects were determined, a Duncan test was used to separate differences between treatment means at the 99% (*p* < 0.01) level of confidence, applying SPSS v27 software (SPSS Inc., Chicago, IL, USA). Correlations between variables with the Pearson's correlation coefficient and Principal Component Analysis (PCA) with parallel analysis as component selection method were applied on growing media, plant growth and biomass production variables using Prism 9.0 for MacOs (GraphPad Inc., Dotmatics, Boston, MA, USA). A biplot-based PCA was carried out using the first two PCs, providing a faithful two-dimensional representation of the relationship (clusters) between GM having similar chemical and biochemical properties according to DWV.

# **3. Results**

# *3.1. Physiological Responses of Plants during Vegetative Growth*

The net  $CO<sub>2</sub>$  assimilation rate (Pn), and transpiration rate (E) decreased in response to the increasing TS concentration in the GM with detrimental effects more pronounced for WF:TS treatments (Table [3\)](#page-6-0).

**Table 3.** Effect of growing medium (GM) and applied daily water volume (DWV) on plant gas exchange parameters and leaf area (cm<sup>2</sup>) of potted cherry laurel.





<span id="page-6-0"></span>**Table 3.** *Cont*.

Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different. ns = non-significant; \* significant at *p* < 0.05; \*\* significant at *p* < 0.01. Sub-stomatal CO<sup>2</sup> concentration (Ci). Water use efficiency of photosynthesis (WUEPn). Transpiration rate (E). Stomatal conductance (Gs). Net  $CO<sub>2</sub>$  assimilation rate (Pn).

Furthermore, the addition of 50% *v*/*v* TS reduced stomatal conductance (Gs) in CF:TS50 and WF:TS50 by 63.7 and 68.4%, respectively, compared to the control. Moreover, it is worth noting that significant differences among the physiological parameters were recorded in plants treated with different DWV (Table [3\)](#page-6-0). Specifically, under reduced DWV, Pn, Gs, and E averaged 12.9, 16.5, and 14.3% lower than those recorded in DWV 250. However, such reductions appeared more evident for mixes containing peat. Indeed, in the hottest season TS25 and TS50 combined with PE were able to provide some buffering capacity to reduce water supply but did not enhance photosynthesis parameters (Pn, Gs and WUE) as much as expected. There were no significant differences in WUE and Pn among GM and DWV.

The color of the leaves showed highest Chroma values in WF:TSs (11.1 and 11.6 for WF:TS25 and WF:TS50, respectively, vs. 7.7 for PE grown plants; Table [4\)](#page-7-0) and were mainly associated to the b\* component more shifted towards yellow. However, these differences clearly decrease over time, with values ranging from a minimum of 7.7 and a maximum of 8.5 among all tested GM.

Small variations in Chls and carotenoids were observed among all sediment-based GM, while in control plants Chl values were found to be higher and carotenoid content was lower. Reduced DWV had a negative effect on Chls and a positive effect on carotenoids. Results of oxidative stress by MDA analysis confirmed this trend (Table [4\)](#page-7-0).

Tissue nutrient concentrations (Table [5\)](#page-7-1) were found to be extremely variable, with P, K, Ca, and Mg being the predominant leaf macronutrients. Higher P contents and clearly lower Mg were detected in the sediment-based media containing WF compared to those mixed with PE and CF. In general, Ca and K showed an opposite trend, being Ca less abundant and K more copious in mixes containing PE alone or combined with TS.

<span id="page-7-0"></span>**Table 4.** Effect of growing medium (GM) and applied daily water volume (DWV) on chlorophyll (Chls) and carotenoid contents (ug/mg dry weight), oxidative stress (MDA) (mM g−<sup>1</sup> dry weight) and Chroma index of potted cherry laurel.



Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different. Chlorophylls (Chls). Oxidative stress (MDA). ns = non-significant; \* significant at *p* < 0.05; \*\* significant at *p* < 0.01.

<span id="page-7-1"></span>Table 5. Effect of growing medium (GM) on leaf micro and macro nutrient content (mg kg<sup>-1</sup> dry weight) of potted cherry laurel.



Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different. ns = non-significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

Concerning microelements (Table [5\)](#page-7-1), higher Cu, Fe and Zn were measured in plants cultivated on the sediment-based growing media blended with WF. Moreover, Cu increased with DWV 250, while Fe with DWV 200. Mn was much more variable, with control plants exhibiting the highest concentration being 21 times the lowest found in plant grown on CF:TS50. As, Be, Cd, Co, Cr, Mo, Ni, Pb, Se, Ti and V were not detected in plant leaf samples.

## *3.2. Plant and Growing Media Performance at the End of Growing Cycle*

## 3.2.1. Properties of Growing Media

The main physicochemical properties of the tested growing media are reported in Table [6](#page-9-0) and Supplementary Materials Table S1. Growing media were characterized by a chemical, physical and biochemical approach.

Numerous changes in physical and chemical properties of the tested substrate mixes were recorded at the end of the growing cycle between the considered GM and DWVs. The BD had the lowest values in the control (0.42 g cm<sup>-3</sup> on average), while in PE:TS, CF:TS and WF:TS mixes it reached values in the range of 0.64–0.79 g cm<sup>−3</sup>, 0.78–0.83 g cm<sup>−3</sup>, and 0.71–0.82 g cm<sup>-3</sup>, respectively. There were not significative differences for particle density (2.49 g cm<sup>-3</sup>). As expected, differences in BD were reflected in TPS; with the highest values in Pe (84.2% and 80.2% in DWV 250 and DWV 200, respectively). Moreover, the reduction in water supply significantly reduced BD in both TS25 and TS50 mixes, while an opposite effect was noticed in the control. Even though the GM receiving reduced water supply did not show significant differences in TPS; higher values in WC, AC and AW were recorded in DWV 200 compared to DWV 250.

The pH was much lower in the control (PE) and higher in the WF:TS mixes; it slightly decreased in all GM receiving less water intake (DWV 200). GM\*DWV interaction had a significantly influence on EC. Values increased with reduced water supply in all blends containing peat, while decreased in all CF:TS and WF:TS mixes; although only in CF:TS50 and WF:TS25 were these differences so large as to be statistically important. Conversely, N-NO<sub>3</sub><sup>-</sup> and N-NH<sub>4</sub><sup>+</sup> exhibited an opposite clear pattern: N-NO<sub>3</sub><sup>-</sup> increased with reduced irrigation, with the only exception of PE, while N-NH<sub>4</sub><sup>+</sup> was generally higher when plants were irrigated with DWV250 compared to DWV200.

TN content was much higher in the control substrate (PEs = 0.56–0.66%) and in the presence of high percentage of PE (PE:TS25s =  $0.35-0.36\%$ ) and WF (WF:TS25s =  $0.26-0.29\%$ ), while DWV did not affect TN content. TOC followed a similar trend as TN. TP contents were in the 500–800 mgP  $kg^{-1}$  range, with extreme values found in PEs. TP increased under low DWV, except for mixes containing WF, and such rise was particularly noticeable in the control.

Concerning substrate enzyme assay, phosphatase activity (Acid-P) followed the same trend of TOC and TN. Enzymatic activity linked to C-cycle (β-Glu) showed significantly lower values in TS50s compared to TS25s (336.2 vs. 523.8 µmol  $\rm{g^{-1}\,h^{-1}}$ ), while the effect of DWVs was less evident, even though significant. Butyrate esterase (B-Est) activity behaved similarly to β-Glu. On the other hand, the enzyme activity related to S cycles (Aryl-S) content was minimum in the control (PEs) compared to all TSs; furthermore, Aryl-S content was not influenced by DWV.

Regarding nutrients, levels of K and Na were highest in PEs and lowest in TS50s, while Ca content showed an inverse trend, being positively correlated with the presence of the TS. Mg followed the trend WF:TSs > CF:TSs-PE:TSs > PEs. No significant variations in macronutrient contents were detected between the two applied water volumes.

In general, the content of heavy metals (Zn, Pb, Fe, Cr, Cu and Ni) was highest in WFs, lowest in PEs, and consistently increased with increasing TS in the mixtures (TS50s), regardless of DWV.



**Table 6.** Growing media properties at the end of the growing cycle.

<span id="page-9-0"></span>Air Content (AC) (% *v/v*). Available water (AW) (% *v/v*). Electrical conductivity (EC) (dS m<sup>-1</sup>). Nitrate nitrogen (N-NO<sub>3</sub><sup>-</sup>) (mg N kg<sup>-1</sup> dw<sup>-1</sup>). Ammonium nitrogen (N-NH<sub>4</sub><sup>+</sup>)<br>(mg N kg<sup>-1</sup> dw<sup>-1</sup>). Total phosphoru dw<sup>−1</sup> h<sup>−1</sup>). Mean separation within columns by Duncan's multiple range test. Means followed by different lowercase letters are significantly different within DWV. Means followed by different capital letters are significantly different between GM. ns = non-significant; \*\* significant at *p* < 0.01.

# 3.2.2. Plant Growth and Biomass Production

Results of MPH, NVS, LVS and NEL of cherry laurel plants cultivated on the different sediment-based GM are shown in Table [7,](#page-10-0) while plant biomass mean values are reported in Table [8.](#page-10-1) The GM had a clear effect on plant height, shoot number, trunk diameter, and final plant biomass production, while DWV was without effect on all areal traits, and GM\*DWV significantly influenced only some growth parameters of cherry laurel during the harsh growing season (July–August). More specifically, MPH and NVS strongly increased over time when plants were grown on PE:TS media, reaching values of 103.2 cm and 102.7 cm MPH, and emitting totally 24.9 and 28.3 NVS in March 2022, on PE:TS25 and PE:TS50, respectively (Table [7\)](#page-10-0).

<span id="page-10-0"></span>**Table 7.** Effect of growing medium (GM) and applied daily water volume (DWV) on maximum plant height (MPH), number of vegetative sprouts (NVS), length of vegetative sprout (LVS), number of fully expanded leaves on vegetative sprouts (NEL), and base stem diameter (BSD) of potted cherry laurel.



Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different. ns = non-significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

<span id="page-10-1"></span>**Table 8.** Effect of growing medium (GM) and applied daily water volume (DWV) on plant biomass in dry weight (DW) and relative growth rate (RGR) of potted cherry laurel.



Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different. ns = non-significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

Growth parameters increased linearly but slower with CF:TS reaching 87.5 to 94.8 cm in height with the emission of 23.5–27.0 new sprouts. Plant grown on WF:TS mixes had a more compact shape and significantly lower DW biomass (areal + roots) compared to the control plants. Specifically, in plants grown on WF:TS the plant height, shoot number, and total plant DW reduction in comparison to control plants was 25.8–27.7%, 46.2–52.3%, and 27.2–27.3% (Tables [7](#page-10-0) and [8\)](#page-10-1). The lowest biomass production observed was mainly attributed to the reduction in both stem weight and total leaf area (Table [8\)](#page-10-1).

Interestingly, a GM\*DWV interaction was noted immediately after the extremely hotdry summer growth period, resulting DWV 200 detrimental for plant growth only when applied on PE alone or PE in combination with TS (Table [9\)](#page-11-0). The effect of water deficit appeared more evident on plants grown on PE alone and PE:TS25.



<span id="page-11-0"></span>**Table 9.** Effect of growing medium \* daily water volume interaction on maximum plant height (MPH), number of vegetative sprouts (NVS), length of vegetative sprout (LVS), number of fully expanded leaves on vegetative sprouts (NEL), and base stem diameter (BSD) recorded at the end of the hot-dry summer period (September) of potted cherry laurel.

Mean separation within columns by Duncan's multiple range test. Means followed by different letters are significantly different. ns = non-significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

## 3.2.3. Principal Component Analysis (PCA)

The PCA was performed with both GM and plant growth parameters recorded at the end of the growing cycle. To reduce the number of variables, a correlation matrix was carried out (Figure S2); then, parameters having a coefficient correlation greater than 0.90 were removed. Going more in detail, pH was highly correlated with BD, Pb, Fe, Cr, Cu, and Ni; BD with TPS; AW with WC; TN with TOC and Acid-P activity; β-Glu with B-Est activity. Indeed, pH, TN and  $β$ -Glu were selected as proxies of other parameters. PCA isolated two principal components (PCs) (total explained variance 49.7%). The first PC (34.4%) included pH, AW, TN, Aryl-S, Zn, K, Na, the most important growth (MPH and NVS) and areal biomass parameters (SDW, LDW, TDW). N-NH<sup>4+</sup>, K, AC, RDW and LVS were significant on PC2 (15.3%). Figure [1](#page-12-0) provides the biplot of the PCA obtained using the first two PCs. This biplot provides a faithful two-dimensional representation of the relationship (clusters) between GM having similar chemical and biochemical properties according to DWV.

<span id="page-12-0"></span>

Figure 1. PCA biplot (left) and principal components (PCs) and component loadings (right). DWV 250 Yellow point; DWV 200 Purple point. \* Variables with component loading used to interpret the 250 Yellow point; DWV 200 Purple point. \* Variables with component loading used to interpret the PCs: threshold level 0.60. Electrical conductivity (EC). Nitrate nitrogen (N-NO<sub>3</sub><sup>-</sup>). Ammonium  $t_{\rm eff}$  (N-NH<sub>4</sub>+). Air content (AC). Available water (AM). Total phosphorus (TP). Total pitrogen nitrogen (N-NH<sub>4</sub><sup>+</sup>). Air content (AC). Available water (AW). Total phosphorus (TP). Total nitrogen (TP). Total nitrogen (TN). Glucosidase activity (β-Glu). Arylsulfatase activity (Aryl-S). Total zinc (Zn). Total magnesium (Mg). Total potassium (K). Total sodium (Na). Total leaf area (TLA). Root dry weight (RDW). Stem dry weight (SDW). Leaf dry weight (LDW). Total dry weight (TDW). Maximum plant height (MPH). Number of vegetative sprouts (NVS). Length of vegetative sprout (LVS). Number of fully expanded leaves on vegetative sprouts (NEL). Base stem diameter (BSD).

The two controls (PEs) were in the top-left on the plot, while PE:TS and CF:TS mixes were in the middle-bottom of the plot; all WFs were in the top-right of the quadrant. Being located in the left part of the plot, PEs resulted positively associated with AW, TN, TLA, SDW, LDW, TDW, MPH and NVS. WF:TSs, being located in the top-right of the plot resulted greatly influenced by pH, Zn, Aryl-S, and N-NH<sub>4</sub><sup>+</sup> and negatively correlated to AC, LVS, RDW, and to the most important growth (MPH and NVS) and areal biomass parameters (SDW, LDW, TDW). CF:TS and PE:TS, being located in the middle-bottom of the plot, resulted mainly influenced by significant parameters on PC2 (namely, AC, LVS and RDW), instead of PC1. In general, each GW\*DWV250 combination was shifted upwards along PC2 when compared to the respective GM\*DWV200 interaction. The GM containing the greatest amount of peat (PE and PE:TS25) were those that showed the greatest variations on both axes according to the applied DWV, while all further mixes revealed limited deviations.

## **4. Discussion**

## *4.1. Growing Media Effect*

## 4.1.1. Comparison between Peat- and Sediment-Based Growing Media (PE vs. TSs)

PE (composed by peat and pumice) had suitable physical and chemical properties as expected [\[29\]](#page-17-12). On the other hand, the main disadvantages of the sediment obtained by the phytoremediation process was a high bulk density; thus, it cannot be considered as an appropriate substrate by itself but might be used as component for potting mixtures in many peat-based cultivations (Table [1\)](#page-2-0). Previous studies reported that up to 50% of peat replacement by TS did not affect lettuce [\[17,](#page-17-0)[30\]](#page-17-13), strawberry [\[12,](#page-16-10)[14\]](#page-16-11) and pomegranate [\[13,](#page-16-12)[31\]](#page-17-14) growth and yield. However, in these contexts the scientific opinions have also to consider the possible hazards associated with sediment-based substrates, as sediments can accumulate toxic compounds and transfer them in plants and food products. Furthermore, sediments have been discussed as additives or substitutes for peat in safe growing media for evergreen shrub nursery production [\[10\]](#page-16-8). Thus, an ideal balance of TS and standard commercial matrixes must be found to improve the use of TS as a soilless growing media for ornamental plant production in the future.

In general, as expected, based on the characteristics of the sediment, all the TS-based GM showed physical properties that did not meet the optimal value proposed by Raviv [\[32\]](#page-17-15) for growing media, although capable of supporting plant growth. All the TS-based GM (TSs) had a high BD and a low TPS, which in a prior study on cherry laurel propagation was found to prevent root penetration and expansion [\[19\]](#page-17-2). Moreover, TSs displayed high EC and pH, high Ca and metal content, and low levels of AW. (Tables S1 and [6\)](#page-9-0) with respect to PE. AC resulted the highest in PE at DWV 250, and in WF:TS25 at DWV 200. As a consequence, control plants had a higher development in PEs, as evidenced in the PCA (Figure [1\)](#page-12-0) by their clear separation in the top-left quadrant and its positive correlation with growth traits. In addition, plant growth appeared to be greatly influenced by the macronutrient content (TN, TOC, Ca, TP) and AW of GM, as demonstrated by their positive correlation with plant growth parameters (Figure [1\)](#page-12-0). Moreover, all TSs presented higher values of pH and higher content of heavy metals with respect to the control (Tables S1 and  $6$ ), that might cause an inhibition in root growth that alters water balance and nutrient absorption, thereby affecting their transportation to the aboveground plant parts and ultimately decreasing biomass accumulation [\[33\]](#page-17-16). However, at this regard it is worth mentioning that heavy metals in the sediment-based growing media were in line with the European Commission and Italian legislation for fertilizers [\[34](#page-17-17)[,35\]](#page-17-18).

Acid-P, Aryl-S, β-Glu, and B-Est activities, enzymatic activities linked to P, S, C cycles and overall microbial activity were affected by the quantity of TS presented in the GM (Tables S1 and [6\)](#page-9-0), given that microorganisms optimally shape their metabolite production to acquire relatively limited nutrient resources [\[36](#page-17-19)[,37\]](#page-17-20). Indeed, PEs differentiated to other GM types by the highest acid phosphatase levels and lowest pH. These results were in line with correlations reported by Paillat et al., [\[38\]](#page-17-21) on microbial enzyme activities on peat, coir, and bark under organic fertilization. In fact, peatland is renowned for high phosphatase activity, indicating P rather than N limitations under these conditions [\[39\]](#page-17-22), due to low level of P availability in peatland [\[40\]](#page-17-23). Instead, β-Glu and B-Est were significantly lower in all GM media having a larger TS fraction, given that microbial activity and carbon cycles are usually strictly correlated [\[41\]](#page-17-24). The trend of Aryl-S was opposite, with higher values in T50s compared to T25s, that may be ascribed to an increase demanding of S by microorganisms, probably due to the high level of inorganic sulphur in the sediment [\[41,](#page-17-24)[42\]](#page-18-0).

The leaf photosynthetic rate was higher in PE (Table [3\)](#page-6-0), probably due to the most favorable substrate physicochemical properties that ultimately increased water transport, and consequently enhanced photosynthetic rate. On the other hand, the more sever effects exerted by TS50 on the photochemistry of cherry laurel were probably due to a direct effect of TS induced strain and were consistent with the lowest RGR. In fact, in plants grown under TS50, conversely to control plants the internal  $CO<sub>2</sub>$  concentration was increased in sub-stomatal cavities (CI of TS25 did not differ compared to control). This supported the hypothesis that, besides stomatal closure, the stronger photosynthetic decline associated with the addition of 50% TS in the GM was also due to possible biochemical limitations, as indicated by the reduction in the electron transport rate.

The lower chlorophyll content of plants grown on all TS mixes could be attributed by a lower N uptake by plants from these GM compared to the control (Table [4\)](#page-7-0). On the other hand, total carotenoids amount increased on Chls basis. Carotenoids has been suggested to be related to a decrease in total Chls as a photoprotective response to compensate the presence of impaired regulations of photosynthesis Khuong et al. [\[43\]](#page-18-1). However, MDA concentration was similar in all plants regardless of the GM and DWV (Table [4\)](#page-7-0), confirming that plants were not exposed to oxidative stress, being the MDA the end-product of lipid peroxidation in the presence of oxidative stress [\[44,](#page-18-2)[45\]](#page-18-3).

4.1.2. Comparison among Sediment-Based Growing Media Mixed with Different Matrices (PE:TS vs. CF:TS vs. WF:TS)

In general, peat associated to TS (both PE:T25 and PE:TS50) constituted a valid growing media for containerized cherry laurel production. Cherry laurel grown in both PE:TS blends had a higher final MPH than those grown on CF:TS; however cumulative NVS and final TDW did not statistically differ between them (Table [7\)](#page-10-0). In fact, these blends were found quite close together in the plot graph (Figure [1\)](#page-12-0), evidencing that both PE and CF matrices can be successfully used in combination with TS for *P. laurocerasus* nursery shrub production, since they provide growth indexes which are comparable to the control. Additionally, the physical properties of CF and PE seemed very similar. On the other hand, plants grown on mixes containing WF behaved very different, regardless of the added TS percentage. Such behavior was highlighted in the PCA, where these mixes separate quite clearly from all the others, being in the rightmost part and negatively correlated with the plant growth parameters.

The high value of pH, Zn and enzyme activities as well as the reduced availability of water (AW) and the higher values in AC in WFs might be concomitant reasons for the reduced plant development, as highlighted by the low values of LVS and RDW (Tables S1 and [6\)](#page-9-0). In this regard, it is noteworthy that high pH levels during rooted cutting establishment caused decreases in leaf expansion and total leaf area, thus limiting plant growth and lowering final DW yields [\[19\]](#page-17-2). Since the pH of WF:TSs lowered by the end of the present growth trial (at an average pH 7.1–7.2) (Table [6\)](#page-9-0), and cherry laurel grown in WF:TS did not significantly differ in the number of leaves (Table [7\)](#page-10-0), it is possible that the initial exposure of the plants to high pH levels in WF caused reduced leaf areas, thereby preventing future growth by lowering water and mineral uptake and rates of photosynthesis. In addition, higher levels of Zn in WF substrates might have inhibit some plant metabolic functions, affecting and limiting growth of both root and shoot, as discussed by Asati et al. [\[46\]](#page-18-4).

GM significantly affected photosynthetic rate (Table [3\)](#page-6-0). Gas exchanges evidenced a significant decline in net photosynthesis, stomatal conductance and transpiration rate for plants grown in CF:TS as well as WF:TS compared to PE:TS. Consistently with the data obtained for plant growth, TS25s lowered Pn rate less than TS50s. The stronger decline of photosynthesis observed under TS50 treatment was not accompanied by an equally strong reduction in stomatal conductance, this latter, occurring at the same extent in both the CF and WF treatments. In this regard, it has been reported that a decreased leaf photosynthesis might be attributed to nitrogen immobilization caused by the high  $C/N$  ratio in WF [\[47\]](#page-18-5), being photosynthetic rate positive related with leaf nitrogen content. However, in our study leaf nitrogen analysis was not contemplated. Moreover, C:N ratio in WF was not much higher than those found for PE and CF, thus it is not to blame for nitrogen immobilization. As a matter of fact, the easy and rapid microbial decomposition of WF versus PE and CF might be considered as the main driver of nitrogen flow in the GM [\[48](#page-18-6)[,49\]](#page-18-7).

WF appeared negatively related to enzyme activities (Tables S1 and [6\)](#page-9-0). These weak activities and investment in enzyme production in WF:TS50 might indicate the presence of labile organic compounds, containing the carbon and nutrients need for microbial growth [\[50\]](#page-18-8). The synthesis of enzymes represents an important investment in energy for microbes and might not be always necessary [\[51\]](#page-18-9). Thus, microbes in WF might be more initially limited by nutrients than C, as revealed by low N-NH<sub>4</sub><sup>+</sup>. Initial high PE N-NH<sub>4</sub><sup>+</sup> is very likely to be related to the fact that microbes convert organic N compounds into organic forms by ammonification realizing mostly nitrogen as  $N-NH_4^+$ .

## *4.2. Irrigation Effect*

The applied DWV exerted a considerable influence on N profile of substrate mixes, plant growth and photosynthetic rate, especially when peat was present in the mixture.

As expected, the different water regime affected the trend of N-NO<sup>3–</sup> and N-NH<sup>4+</sup> in all GM (Table [6\)](#page-9-0). More specifically, N-NO<sub>3</sub><sup>-</sup> was generally lower under DWV250 in all

GM, while an opposite trend was observed for N-N $\rm H_4^+$ , with a lower value in DWV200. This trend was probably due to the highest AC in DWV200, suggesting a better aeration of the substrates under low water regime, which therefore allowed greater nitrification [\[52\]](#page-18-10), given that N-NH<sub>4</sub><sup>+</sup> and AC resulted inversely correlated on PC2. On the other hand, the loss of nitrate could also be due to a higher nitrate leaching and/or plant absoprtion in DWV250, given that the trend of N-NO<sub>3</sub><sup>-</sup> was not correlated neither with N-NH<sub>4</sub><sup>+</sup> or to AC (Figure S2). It is worth of notice that GM with reduced DWV presented a higher level of AC and AW; this fact was probably due to the change in macro and microporosity of the growing media during cherry laurel growth [\[53\]](#page-18-11).

Average values of Gs were lower for droughted cherry laurel plants compared to regularly irrigated plants grown in the same media, except for PE:TS mixes (Table [3\)](#page-6-0). However, the significantly interaction between GM and DWV treatment for both Gs and Pn demonstrated that the induced drought affected plant growth in each media differently. Droughted plants grown in peat and 50% TS had significantly higher Gs than those grown in WF:TS50, with those grown in CF:TS50 being statistically different from either. The good leaf photosynthetic rate in PE:TS50 under reduced water availability could be explained by increased Gs, which may be associated with the increased soil water holding capacity. The only significant GM\*DWV differences in E were found for PE, PE:TS25 and CF:TS25, which showed higher values under higher water availability compared to water deficit, thus reducing crop water use efficiency. Nevertheless, the MDA determination regarding the oxidative stress status in the leaves indicated that plants had a similar lipid peroxidation level in all treatments.

# **5. Conclusions**

Final plant development, biomass and chlorophyll content of cherry laurel grown on PE:TS and CF:TS mixes showed comparable trends of control plants grown on peat, demonstrating the physical and chemical fertility of the sediment-based GM. The lower final biomass of plants grown on WF:TS mixes, especially under reduced DWV, was most likely to be related to the combined effect of the properties of the used matrices (high values of pH, BD, N-NH<sup>+</sup>, heavy metals, as well as K e Ca compared to Mg) and reduced water availability. However, despite the differences found in the tested GM (essentially for MPH, NVS and TDW), *P. laurocerasus* development in blends containing PE as well as CF and WF were consistent with 2-year-old nursery grown cherry laurel quality standards reported for marketing category of 7-L pot grown plants (60–80 cm plant height) (Figure S3). This therefore indicates productions relevant to commercial standards for this ornamental crop grown in these treatments, showing that TS could still be used as an environmentally media replacement, even though TDW in CF and WF mixes was 5.7–27.3% lower than in PE:TSs.

Regarding water supply, it has been confirmed by many researchers that water stress leads to growth reduction, which was reflected in plant height, leaf area, dry weight, and other growth functions. Indeed, during the critical summer period some growth reductions were evidenced in control plants and were supposed to be related essentially to the hydrophobic properties of peat. Nevertheless, in our study treatment of 20% water reduction did not affect final plant growth parameters. These results, therefore, suggest that the use of NBS-sediment-based growing media can allow for growing ornamental shrubs with less water, which is relevant today due to water scarcity and climate change. This matrix of marine origin, mixed with peat, coconut fiber, wood fiber and pumice, showed adequate physical and chemical properties over time and allowed cherry laurel plants to meet high-quality standards. Our data strongly support the observation that sedimentbased substrates can be successfully used for growing a number of ornamental plants of the assortment offered by the nursery industry sector among which shrubs, hedges and privacy screens (for instance *Berberis thunbergia*, *Buxus sempervirens*, *Elaeagnus ebbingei*, *Escallonia floribunda*, *Euonimus japonicus*, *Forsythia X intermedia*, *Hibuscus siryacus*, *Hydrangea quercifolia*, *Ilex aquifolium*, *Ilex cornuta*, *Ligustro ionandrum*, *Nandina domestica*, *Nerium oleander*, *Paeonia*

*sinensis*, *Pyracantha coccinea*, *Raphiolepis umbellate*, *Syringa vulgaris*, *Viburnum opulus*) play a predominant role.

**Supplementary Materials:** The following supporting information can be downloaded at: [https://www.](https://www.mdpi.com/article/10.3390/agronomy13010092/s1) [mdpi.com/article/10.3390/agronomy13010092/s1,](https://www.mdpi.com/article/10.3390/agronomy13010092/s1) Figure S1. Rooted *Prunus laurocerasus* cv. 'Novìta' plants from a previous trial on cutting propagation during 2020; Table S1: Growing media properties at the end of the growing cycle; Figure S2: Pearson correlation coefficient (r) matrix and heatmap for the 38 considered GM and growth parameters; Figure S3. Two-year-old cherry laurel plants grown on sediment-based growing media.

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