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Disentangling pH and Salinity Effects in Biochars Used as Peat Substitutes: Insights from Water Washing and Tomato Plant Growth Responses

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Abstract

This study evaluated the effects of water washing on the properties of biochars produced from tomato greens (TB) and vineyard pruning (VB), and examined how these changes influence plant performance in biochar–peat substrates. A 31-day pot experiment was conducted using tomato plants (*Solanum lycopersicum* L.) grown on biochar/peat mixtures at a 60:40 (*v*/*v*) ratio. Fresh biochars (TB 0, VB 0) and biochars subjected to one (TB 1, VB 1) or two (TB 2, VB 2) washings were assessed. Washing substantially reduced pH from 10 to 9 in both materials. The electrical conductivity (EC) and salt content of TB 0 (9147 $\pm$ 96 µS cm^{-1}) were markedly higher than those of VB 0 (1539 $\pm$ 33 µS cm^{-1}). Successive washing effectively lowered EC to 1019 $\pm$ 18 µS cm^{-1} and 75.4 $\pm$ 4 µS cm^{-1}, respectively. Plant performance improved significantly as salinity decreased. Statistical analyses indicated a stronger influence of EC than pH on plant growth. Reduced germination was primarily associated with osmotic stress caused by elevated concentrations of soluble salts, particularly Na, K, Ca, Cl, and S. Biomass production was negatively correlated with these ions. Only Ca and K exhibited significant negative relationships, suggesting that nutrient antagonism may also have contributed to growth inhibition. These findings demonstrate that water washing is an effective strategy for enhancing the horticultural suitability of salt-rich biochars. Salinity, rather than alkalinity alone, appears to be the principal constraint limiting their use as peat substitutes. Washing therefore broadens the potential application of salt-rich green waste-derived biochars in horticultural substrates, supporting peat conservation and circular-economy approaches to organic waste valorization.

Keywords: peat replacement; nutrient excess in biochar; green waste recycling; plant health; circular economy

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1. Introduction

Biochar is a carbonaceous material obtained from biomass, typically derived from agricultural or biowaste, through heat treatment under pyrolysis conditions to prevent rapid oxidation and conversion to CO_2 [1]. Besides its common application in soil amendment

and improvement, it has also been discussed as a peat substitute in horticulture thanks to its unique properties [1–4]. Promising results were published by García-Rodríguez et al. [5], showing that in semi hydroponic cultures of lettuce, using substrate mixtures of vermiculite, peat and biochar with a biochar/peat ratio of 30:40 yields better plant growth and nitrogen use efficiency than the mixture with only vermiculite and peat. Comparable findings were also reported by Rathnayake et al. [6] and Massa et al. [7] indicating that sawdust and popular wood-derived biochar addition to planting substrates at concentrations of 25 to 50% and 20% (*v*/*v*), respectively, can increase plant performance.

However, other studies revealed less encouraging findings suggesting that some biochars have some counterproductive characteristics, reducing plant growth [8,9]. One particularly problematic aspect of many biochars, especially when applied in horticulture, is their high pH, which can result in the insolubility of many plant nutrients [8,10–12]. Commonly associated with high alkalinity is a high electrical conductivity (EC), indicating elevated contents of salts which can cause osmotic stress for plants [13]. Often, such stress can be avoided by limiting the amount of biochar added to the peat substrate to concentrations that only slightly increase pH and EC [5].

In a previous study, Nocentini et al. [3] tested the feasibility of replacing peat in tomato cultivation substrates with biochars produced from chitin, peat, rice husks and tomato greens. They confirmed the large impact of biochar composition and properties on plant performance and reported that replacing peat by 60% (*v*/*v*) with chitin-derived biochar significantly increased plant dry mass production relative to the pure peat substrate. This effect was attributed to the high N content of the biochar, which supplied this essential nutrient during the early stages of seedling development. In contrast, the addition of biochar derived from tomato greens inhibited seed germination and, when germination did occur, significantly impaired plant development. Statistical analysis indicated that this negative effect could not be solely explained by the potential presence of phytotoxic compounds or elevated pH values but was also likely related to high salt concentrations in general, and particularly to elevated levels of sodium (Na^+) and potassium (K^+) ions. While the first affects the osmotic pressure, the latter may be related to the phenomenon of ion antagonism where the presence of one element limits the adsorption of others [14].

Despite the growing interest in recycling green waste as a sustainable approach in the frame of circular economy by its transformation into biochar and subsequent use in horticulture, the understanding of how biochar affects plant growth is still limited [8,15]. Therefore, the goal of the present work was to fill this research gap by identifying key players responsible for the negative impact of alkaline biochars on plant performance. Based on the first results published by Nocentini et al. [3] revealing a high EC of 12.5 mS cm^{-1} for a biochar produced from tomato greens at 400 °C, we hypothesize that:

(i) The salt composition of biochars in biochar-containing planting substrates affects germination and plant performance more detrimentally than alkalinity alone.
(ii) Removing access salt from salt-rich biochar by simple washing with water can turn such biochars, that were unsuitable for horticulture, into products with the potential to be recycled as peat replacements in planting substrates even at concentrations as high as 60% (*v*/*v*).

To test these hypotheses and to disentangle the effect of pH on plant performance from those of salinity, we selected two biochars with comparably high pH of around 10 but different ECs and different ion compositions and tested their performance as peat substitutes in planting substrates. The first biochar was derived from tomato greens as a representative of biochars with high EC, and had Na^+ or K^+ concentrations above 4 g kg^{-1} and 60 g kg^{-1}, respectively [3]. The second originated from the pyrolysis of vineyard pruning residues [5]. As this material tends not to extensively accumulate Na^+

and K^+, the concentrations of those nutrients in this biochar was expected to be low. To reduce salt content and alkalinity of the biochars, they were washed with distilled water. We abstained from using organic or inorganic solvents for the removal of potentially phytotoxic compounds to avoid potential alteration of the organic fraction as much as possible [16,17]. However, decreasing alkalinity and removal of salts with water is not selective and also reduces the content of plant-available nutrients. In addition, it liberates potential adsorption sites at the surface of the biochar that may enhance the competition between biochar and plants for nutrients [18].

Therefore, our study extends beyond a general evaluation of the impact of washing intensity on biochar properties such as organic matter structure, elemental composition, pH, EC and ion contents and includes the investigation of the effects of this approach on plant development. For this purpose, a greenhouse pot experiment with biochar/peat mixtures as substrates was conducted, and the germination rate and growth of tomato seeds and plants were assessed over a period of 31 days. To prevent the beneficial effects of biochar addition from masking the negative impacts of the biochar's high pH and salt content on plant performance, we used substrates containing a relatively high proportion of biochar (60% v/v). According to previous experiments [5], this concentration was sufficient to exceed the threshold at which unwashed biochar derived from pruning residues started to induce plant stress symptoms. In addition, we refrained from applying any nutrient solution to avoid cross-effects. At the end of the experiment, plant biomass, SPAD (Soil Plant Analysis Development) values, and leaf area were measured.

2. Materials and Methods

2.1. Biochar Production, Washing of the Biochars and Characterization of the Biochars and the Peat Substrate

The selected feedstocks to produce biochar were tomato (*Solanum lycopersicum*) greens and vineyard (*Vitis vinifera*) pruning residues. Prior to the production of the tomato greens biochar (TB 0), the feedstock was heated until dry in an oven at 40 °C for a week and after that cut manually into pieces into lengths of <5 cm. This material was pyrolyzed at 400 °C for 3 h using a closed custom-made stainless steel reactor [3], which was filled up to 2/3 of its volume and flashed with N_2 to remove O_2. Afterwards the reactor was placed into a preheated muffler (Hobersal, Caldes de Montbui, Spain) for 3 h. The produced syngas was directed through a stainless steel tube from the reactor to the outside of the muffle and connected to a gas-trap filled with basic vegetal oil solutions. With this approach, we avoided accumulation of volatile compounds and pollutants in the biochar. The vineyard pruning biochar (VB 0) was provided by the company "Caviro-Enomondo" (Faenza, Italy) and obtained after pyrolysis at a temperature of 500 °C [5]. The peat "Sphagnum peat substrate" was purchased from the company "Klasmann-Deilmann GmbH" (Geeste, Germany).

To remove the salt, 25 g biochars were added to 500 mL Elix Water (double distilled water) and shaken in a rotary shaker for 1 h. Subsequently, the water was removed by filtration using laboratory filter paper (Dorsan Filtration, International, Barcelona, Spain) and the biochar was dried at 40 °C (TB 1 and VB 1). Half of the washed biochars were subjected to one additional wash (TB 2 and VB 2). This process was repeated until enough material was obtained for the experiments.

2.2. Characterization of the Peat and the Biochars

The pH (H_2O) of the peat as well as of the untreated and washed biochars were determined in triplicates in a water suspension (1:10 w/v) with a Crison 40 pH-meter (Crison, Alella, Spain). Subsequently, the mixture was filtered (What man Nº2 Filter) to

measure the EC using a Crison EC-meter Basic 30+ and a Crison Basic 20 conductivity meter (Crison, Alella, Spain).

Total carbon (TC) and total nitrogen (TN) of all feedstocks were determined in triplicates via dry combustion using a Flash 2000 elemental micro-analyzer (Thermo Scientific, Bremen, Germany). The mineral nutrients in the original materials were measured using extracts from the samples. The samples were digested with nitric and hydrochloric acid in a DigiPREP Block Digestion System (SCP Science, Montreal, QC, Canada). The nutrients were then analyzed using a inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Varian Inc., Palo Alto, CA, USA) [5]. The chloride (Cl^-) content was determined spectrophotometrically (OMEGA) [19].

Solid-state ^{13}C NMR spectroscopy, applied for a more comprehensive analysis of the chemical composition of the organic matter of the peat substrate and the biochars, was performed using a Bruker Avance III HD 400 MHz spectrometer (Bruker, Madrid, Spain). The dry and homogenized sample material was put into a zirconium rotor of 4 mm OD with KEL-F-caps. The spectra were acquired with the cross polarization (CP) technique and magic-angle spinning of the rotor at 14 kHz. A ramped ^1H-pulse was applied during a contact time of 1, and a pulse delay of 300 ms was employed. The ^{13}C chemical shifts were referenced to tetramethylsilane (0 ppm) and calibrated with glycine (COOH at 176.08 ppm). The chemical composition of the sample was determined by integration of defined chemical shift regions assigned to different C groups [20] as follows: alkyl C (0–45 ppm); N-alkyl/methoxyl C (45–60 ppm); O-alkyl C (60–90 ppm); aromatic C and phenol C (90–160 ppm); carboxyl/amide C and carbonyl C (160–245 ppm). The spinning speed of 14 kHz was not sufficient for complete removal of the chemical shift anisotropy. Therefore, spinning side bands occurred at both sides of the parent signal at a distance equal to the spinning frequency (here, between 225 and 300 ppm, and 0 and 45 ppm). The intensities of these were added to the parent signal between 160 and 90 ppm [20]. The solid-state CPMAS ^{15}N NMR spectrum of TB 0 was obtained with the same equipment, applying a pulse delay of 200 ms and a contact time of 1 ms. Due to the low ^{15}N sensitivity for NMR and the low N content of the sample, 1.2 million scans were accumulated. In spite of this, the spectrum showed a low signal-to-noise ratio that prevented integration and quantification. The ^{15}N chemical shift scale was referenced to nitromethane and was calibrated with ^{15}N-enriched glycine (−346 ppm).

2.3. Greenhouse Experiment and Analysis

The greenhouse pot experiment was performed with peat substrates mixed with either washed or unwashed biochar. In agreement with the focus of our study, we used a biochar/peat mixture ratio of 60:40 (*v*/*v*) [3], which was previously shown to correspond to a concentration of unwashed biochar that is sufficiently high to create considerable plant stress [3,5]. For each mixture, the maximum water holding capacity (WHC) was determined in triplicates. Therefore, 6 g of sample was placed over a Dorsan 102 filter into a funnel and saturated with distilled water. After 2 h percolation, the weight of the wet sample was measured and the amount of water that can be held against gravity was calculated by weight difference.

For the pot experiment, 120 mL plastic containers, previously perforated on the bottom, were filled with 100 mL of the substrate mixtures. In each pot, three tomato seeds (*Solanum lycopersicum*; Roma variety; brand: "HA—Huerto y Jardín") were sowed. Subsequently, the pots were posted into the greenhouse and moistened to 65% of WHC. They were covered with plastic transparent film until most seeds had germinated, and germination rate was monitored throughout the whole experiment. The greenhouse conditions were a 24 ± 2 °C/17 ± 2 °C (day/night) temperature and a 60 ± 10% relative humidity (EL-1-USB

data logger, Lascar Electronics Inc., Erie, PA, USA). The photoperiod was 16 h/8 h with a flux density of photosynthetic photons (Mean PAR) 300–350 µmol m^{-2} s^{-1} (quantum sensor, LI-6400; Li-COR, Lincoln, NE, USA) and a light emission of 9000–10,000 lux (Digital Lux Meter, LX1010B; Carson Electronics, Valemount, BC, Canada). After 12 days, only the strongest seedling was kept in each pot to avoid nutrient competition between shoots. Seeds that had not germinated at that stage were kept until their germination and subsequently removed. Each treatment was realized in quadruplicate. To maintain the moisture of the pot, they were weighed every two days to determine water loss. The lost water was replaced accordingly. The growth of the plants was monitored by measuring the epicotyl length from the soil surface to the insertion point of the highest leaves. The pots were moved around in the tray so that all the plants got the same amount of light and care during the 31 days of the experiment. The plants in the studies were not treated with pesticides or extra fertilizers before or during the studies.

At the end of the experiment, thus 31 days after sowing (DAS), the relative chlorophyll content of the plants was obtained with a SPAD-502 Plus 8 (KONICA MINOLTA, Tokyo, Japan) [21]. Then, leaves were harvested per pot, laid on a white background and photographed to measure the total leaf area using the ImageJ2 program. Subsequently, the aboveground part of each shoot was cut, and its fresh and dry weights were obtained before and after drying at 40 °C for 24 h. The roots were carefully separated manually from the growing substrate, washed with distilled water and dried at 40 °C for 24 h, before being weighed.

The Cl^- content of substrates, before and after the experiment, and the biochars were determined from a aqueous extraction by using a colorimetric method, based on displacement of thiocyanate by Cl^- ions, and was measured with the absorbance microplate reader "Omega SPECTROstar" (BMG LABTECH GmbH, Ortenberg, Germany) [22].

2.4. Statistical Analysis

Statistical analysis was performed using the STATGRAPHICS Centurion XIX software (StatPoint Technologies, Warrenton, VA, USA). Both "one-way" and "two-way" analysis of variance (ANOVA) was applied to determine the significant differences between groups of samples. In addition, a Pearson correlation and two principal components analysis (PCAs) have been carried out to evaluate the effect that each variable has on the results. All comparisons of means were determined using Tukey's honestly significant difference (HSD) and normality of the data was assessed using the Saphiro–Wilk test ($p < 0.05$).

3. Results and Discussion

3.1. Impact of Washing on the Characteristics of the Biochars

The pH values of the washed materials (Table 1) decreased significantly after the first and second washing, indicating the removal of compounds responsible for the alkalinity of the biochars. TB 0 had an initial pH of 9.71 ± 0.08, which reduced to 9.06 ± 0.07 after the first and to 8.62 ± 0.02 after the second wash. Comparably, the initial VB pH of 10.31 ± 0.08 decreased to 9.40 ± 0.06 and then to 8.93 ± 0.19 after the first and second washing, respectively, indicating a loss of soluble cations by the washing process. However, the optimal pH value for tomato growth of between 6.0 and 6.8 was still not reached and plant growth may still be affected by limited by P, Fe and micronutrient availability. The lowering of the pH goes hand in hand with a considerable decrease of EC (Table 1) in particular for TB, suggesting an efficient removal of soluble salts and ionic components, which is in line with the findings of Dunlop et al. [13]. However, no significant correlation between the pH and EC values ($r = 0.29$) was obtained, indicating that factors other than salt concentration determine the pH value of the biochars. The EC values, on the other hand,

showed strong correlations with the contents of Ca (r = 0.80), K (r = 0.91), Mg (r = 0.84), Na (r = 0.99), S (r = 0.99) and Cl^- (r = 0.99). Only modest correlations were observed between pH and Ca (r = 0.51), K (r = 6.3), Mg (r = 0.51), Na (r = 0.37), P (r = 0.51), S (r = 0.15) and Cl^- (r = 0.32).

Table 1. pH, electrical conductivity (EC), total carbon (TC) and nitrogen (TN) contents, the C/N ratios and Cl^- contents of the peat substrate (PE) and biochars derived from tomato green waste (TB) and vineyard pruning residues (VB) before (XX 0) and after the first (XX 1) and second (XX 2) washing with distilled water.

Material	pH	EC (µs cm^{-1})	TC (mg g^{-1})	TN (mg g^{-1})	C/N	Cl^- (mg g^{-1})
TB 0	9.71 ± 0.08 a	9147 ± 96 a	422 ± 11 a *	21 ± 1 b	20	29.6 ± 1.5 a
TB 1	9.06 ± 0.07 b	2863 ± 55 b	462 ± 43 a	31 ± 3 a	15	4.2 ± 0.3 b
TB 2	8.62 ± 0.02 c	1019 ± 18 c	473 ± 31 a	27 ± 2 a	18	0.8 ± 0.1 c
p	***	***	ns	**		***
VB 0	10.31 ± 0.08 a	1539 ± 33 a	409 ± 7 a *	5 ± 0 b	81	0.7 ± 0.1 a
VB 1	9.40 ± 0.06 b	376 ± 39 b	539 ± 12 a	7 ± 0 a	77	0.4 ± 0.1 b
VB 2	8.93 ± 0.19 c	75 ± 4 c	483 ± 55 a	7 ± 1 a	69	0.18 ± 0.03 c
p	***	***	ns	**		**
PE	6.48 ± 0.09	833 ± 4	510 ± 195	20 ± 5	26	0.4 ± 0.2
M	ns *	***	*	***		***
W	***	***	ns	***		***
M × W	***	***	ns	ns		***

* ns: no significant differences. Values followed by different letters in the same column indicate significant differences according to Tukey's test. Levels of significance: "ns" $p > 0.05$. * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$. Statistical differences between material (M), number of washings (W) and their interaction (M × W) are shown at the bottom of the table.

Low EC values are commonly related to nutrient deficiencies, whereas high salt concentrations can cause salt stress. The main players are Na and Cl^- ions in the soil solution, and its adverse effect on plants include primary stresses like osmotic stress and ion imbalance, as well as secondary stresses like oxidative stress and metabolic abnormalities [23]. Tomatoes are moderately salt sensitive with optimal ECs below 2.25 mS cm^{-1}. According to the values in Table 1, this threshold is reached already for the untreated VB but for TB only after the second washing. Tolerable extractable Cl^- concentrations are reported to be in the range of 0.1 to 0.2 mg g^{-1} [24]. For TB 0, the Cl^- content of 29.6 ± 1.5 mg g^{-1} was approximately 15 times higher than this value, and only after two washings, a considerable lowering to 0.8 mg g^{-1} was reached (Table 1). With 0.7 mg g^{-1}, VB 0 only slightly exceeded the value for optimal tomato growth. However, washing two times resulted in an approximation to this optimum.

A comparable pattern was observed with respect to the concentration of total Na (Table 2). Whereas TB 0 shows extremely high concentration of 7.4 mg g^{-1}, this value was reduced to 1.3 mg g^{-1} after two washings. The moderate content of 0.7 mg g^{-1} for VB 0 remained almost unaffected by the treatment.

Table 2. Total contents of nutrients in the fresh biochars TB 0 and VB 0, after the first (TB 1 and VB 1) and second washing (TB 2 and VB 2), and the peat.

Material	Zn (mg g^{-1})	Ca (mg g^{-1})	Fe (mg g^{-1})	K (mg g^{-1})	Mg (mg g^{-1})	Mn (mg g^{-1})	Na (mg g^{-1})	P (mg g^{-1})	S (mg g^{-1})
TB 0	0.2	119.7	0.2	33.5	18.1	0.1	7.4	4.4	13.2
TB 1	0.2	101.5	1.1	18.4	11.2	0.1	2.1	7.2	4.9
TB 2	0.2	69.8	0.5	9.6	10.5	0.1	1.3	6.2	1.8
VB 0	0.2	45.2	2.4	14.4	6.0	0.3	0.7	4.4	0.7
VB 1	0.3	54.1	6.4	9.2	8.2	0.4	0.9	4.0	0.6
VB 2	0.2	56.5	6.8	7.8	8.6	0.4	0.7	4.1	0.6
PE	0.0	14.9	6.1	1.5	1.8	0.2	0.2	0.7	3.1

Concomitant with the loss of salts, a slight but not significant increase in the total carbon (TC) and total nitrogen (TN) occurred, suggesting that loss of organic material caused by the washing may be negligible. Comparable observations are reported in the literature [25,26]. In our study, the TC of TB 0 and VB 0 increased from 422 ± 11 mg g^{-1} and 409 ± 7 mg g^{-1} to 473 ± 31 mg g^{-1} and 483 ± 55 mg g^{-1}, respectively, after two washings. Whereas VB 0 showed a C/N ratio of 81, expected for biochar derived from feedstock containing woody residues, TB 0 had a considerably lower ratio of 20 which is typical for biochar produced from green waste containing organic N compounds. As revealed from ^{15}N NMR spectroscopy, most of this organic N had been transformed into pyrrole-like structures resonating in the ^{15}N chemical shift region between −200 to −260 ppm during the heating process (Figure 1). Note that pyrrole N in aromatic structures can act as an acid. It can be speculated that this group contributes to buffering of the pH in TB 0 to TB 2.

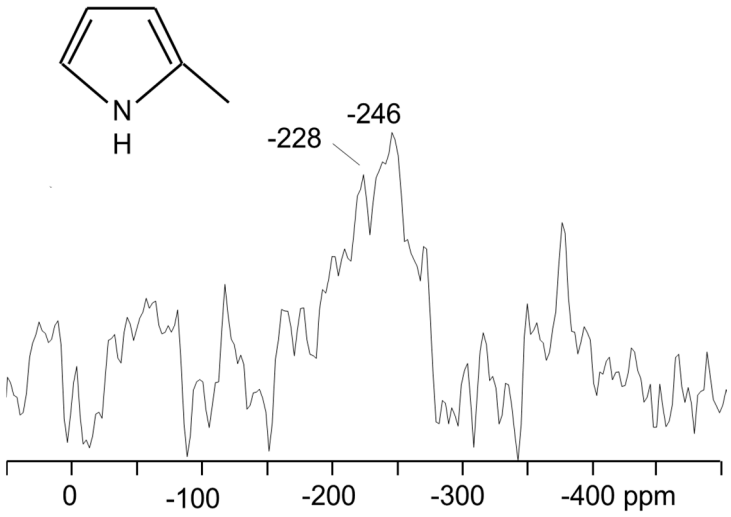


Figure 1. Solid-state ^{15}N NMR spectrum of a biochar derived from tomato greens and produced at 400 °C (TB 0).

The concentrations of As, Cd, Cr, Cu, Ni, Hg and Pb in all biochars were not significantly affected by the washing (Table S1) and are below or close to the minimum threshold of potentially toxic elements (PTEmin) for composts from organic waste in Europe (ENV.A.2./ETU/2001/0024, Annex 2). Zn concentrations were between 0.2 and 0.3 mg g^{-1} and thus still below PTEmax for this element (1.5 mg g^{-1}) (Table 2).

The contents of K and S decreased after washing for all treatments, although it was more pronounced for TB (Table 2). The leaching of these elements during washing is best explained by their high solubility in water [27–29].

Contents of Ca, Mg and Na were considerably higher in TB 0 than in VB 0. Whereas for TB 0, these elements were largely removed by the treatment, washing of VB 0 resulted in a small increase in Ca and Mg levels, most likely caused by a relative enrichment due to the loss of other elements (Table 2) [25,27].

3.2. Characterization by Solid-State ^{13}C NMR Spectroscopy

The solid-state ^{13}C NMR spectra of the fresh biochars shown in Figure 2 confirm the high aromaticity typically observed for such samples (Table 3) [20]. The respective chemical shift regions of phenol C (160 to 140 ppm) and aryl C (140–90 ppm) comprise together 58% for TB 0 and 79% for VB 0 of the total ^{13}C intensity. The aryl C to *O*-aryl C ratio of VB 0 is with 6.3 wider than TB 0 with 5.0, indicating that the aromatic network of VB 0 has less O-substitution than TB 0. This can be explained by the higher production temperature of VB leading to transformation of benzofuran structures into arene units [30,31]. However, as shown in Figure 1, organic N in TB 0 occurs mostly as pyrrole [32]. Its connected C resonates also in the ^{13}C chemical shift region between 160 and 140 ppm. Accordingly, in the spectrum of TB 0 and TB2, such compounds contribute to the intensity of this region.

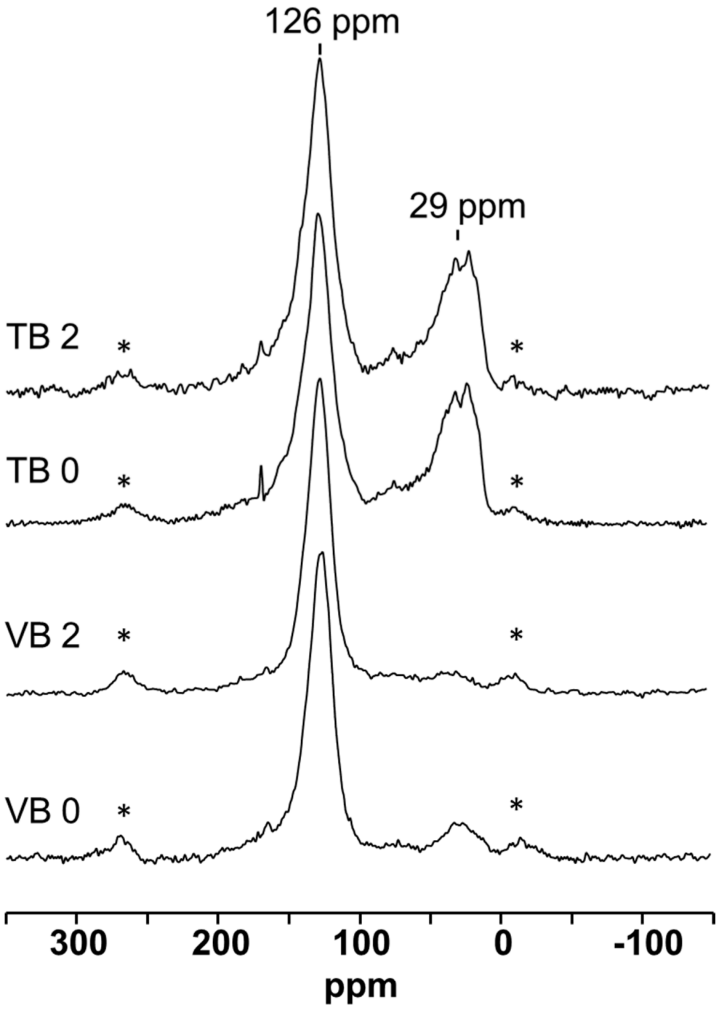


Figure 2. ^{13}C NMR spectra of biochar from tomato green waste (TB) and vineyard pruning (VB) produced at 400 °C and 500 °C, respectively, before (TB 0, VB 0) and after washing two times (TB 2 and VB 2). Asterisks indicate spinning side bands.

Table 3. Relative contribution of different C classes to the total organic C of fresh biochars (TB 0 and VB 0) and the biochars after two washings (TB 2 and VB 2) according to solid-state ^{13}C NMR spectroscopy.

C Group (% of TOC)	TB 0	TB 2	VB 0	VB 2
Carbonyl C	2.5	2.5	1.4	2.2
Carboxyl C	4.3	4.8	4.5	4.2
***O*-aryl C**	9.6	10.1	9.2	9.4
aryl C	47.9	48.2	67.6	70.6
***O*- alkyl C**	10.7	10.8	6.8	7.3
alkyl C	24.9	23.5	10.4	6.3
aryl C/*O*-aryl C	5.0	4.8	7.3	7.5

Lower production temperature and the higher N content of TB 0 compared to VB 0 can also explain the difference in the intensity of the O-alkyl and alkyl C region of their spectra, since increasing pyrolysis temperature is commonly associated with loss and transformation of alkyl C (Table 3). Both in the spectrum of TB 0 and VB 0 a small and narrow signal at 164 ppm was identified and can be assigned to carbonate C.

Following the washing process, the spectra of TB 0 and TB 2 reflect a comparable pattern. This is in line with the observation that the TC content remained unaffected by the washing (Table 1). Thus, it can be concluded that in spite of efficient removal of salts, the overall chemical structure of the organic network of this biochar was not changed. Although the overall pattern of the spectrum of VB 0 was not markedly affected by the treatment, the relative contribution of alkyl C decreases slightly after washing (Table 3). This may have been caused by a small loss of water-soluble alkyl moieties. However, this loss did not lead to a reduction in the TC content (Table 1) and had no major impact on the overall organic structure of this biochar.

3.3. Impact of Biochar Washing on the Peat–Biochar Substrate Characteristics

As expected, mixing the biochars with the peat at a ratio of 60 to 40 (*v*/*v*) decreased the pH of the substrate (Tables 1 and 4). However, with plant growth, the pH of all mixes increased slightly until the end of the experiment. The higher pH values obtained from the same mixes after 31 DAS could have been caused by the relative accumulation of inorganic alkaline biochar compounds caused by the preferential degradation of peat-derived organic matter. However, solid-state ^{13}C NMR spectroscopic analysis of the substrates before and after incubation did not show any increase in the relative aromaticity, typical for the relative preservation of biochar (Table S2). A further possible explanation, though not tested here, is the effect of biochar on microorganisms, stimulating the alkalizing process in the nitrogen cycle, such as denitrification. This process, particularly the reduction of N_2O to N_2, consumes protons (H^+), which could potentially increase the pH of the substrate after 31 days [33,34].

As expected, mixes containing unwashed biochar had higher EC values (TB 0: 4517 ± 398 µS cm^{-1}; VB 0: 751 ± 105 µS cm^{-1}) than mixes containing biochar washed once (TB 1: 1355 ± 32 µS cm^{-1}; VB 1: 433 ± 18 µS cm^{-1}). Mixes using biochar washed twice have the lowest EC values (TB 2: 619 ± 7 µS cm^{-1}; VB 2: 261 ± 13 µS cm^{-1}) (Table 4). The EC decreased slightly in almost all mixes after 31 DAS.

Table 4. pH (at 0 and 31 DAS), electrical conductivity (EC) (at 0 and 31 DAS), water holding capacity (WHC%) and chloride content (Cl^-) (at 0 and 31 DAS) of substrates based on washed and non-washed biochars mixed with peat at 60:40 (*v*/*v*).

Treatment	pH		EC (µS cm^{-1})		WHC (%)	Cl^- (mg g^{-1})	
	0 DAS *	31 DAS	0 DAS	31 DAS		0 DAS	31 DAS
TB 0	8.07 ± 0.08 b	8.66 ± 0.17 b	4517 ± 398 a	3843 ± 533 a	223 ± 16 a	17.9 ± 1.0	19.1 ± 2.3 a
TB 1	8.07 ± 0.01 b	8.80 ± 0.07 b	1355 ± 32 b	1357 ± 88 b	179 ± 11 a	2.7 ± 0.2	3.2 ± 0.5 b
TB 2	8.21 ± 0.02 a	9.27 ± 0.08 a	619 ± 7 c	601 ± 39 c	176 ± 27 a	0.7 ± 0.1	0.39 ± 0.02 c
p	*	***	***	***	*	-	***
VB 0	9.31 ± 0.13 a	10.05 ± 0.06 a	751 ± 105 a	590 ± 22 a	181 ± 12 b	0.6 ± 0.1	0.5 ± 0.9 a
VB 1	8.38 ± 0.16 b	9.50 ± 0.04 b	433 ± 18 b	310 ± 14 b	213 ± 15 a	0.4 ± 0.1	0.3 ± 0.1 b
VB 2	8.49 ± 0.12 b	8.96 ± 0.11 c	261 ± 13 c	250 ± 13 c	127 ± 5 c	0.4 ± 0.1	0.2 ± 0.1 b
p	***	***	***	***	***	-	**
M	***	***	***	***	*	-	***
W	***	***	***	***	***	-	***
M × W	***	***	***	***	***	-	***

* DAS: days after sowing. Values followed by different letters in the same column indicate significant differences according to Tukey's test. Levels of significance: * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$. Statistical differences between material (M), number of washings (W) and their interaction (M × W) are shown at the bottom of the table.

The WHC (%) of the peat/biochar mixes was not significantly affected by the washing of TB 0 and those with VB showed only minor alterations, indicating that the pore structure was, if at all, only slightly affected by the removal of salts through the washing.

The Cl^- contents (mg g^{-1}) of the mixes reflect the efficient removal of this ion with washing since it decreases considerably from TB 0 to TB 2 and showed no major differences for the substrates based on VB 0 to VB 2 (Table 4). Plant growth had no major impact on its content in the substrate after 31 days of incubation except for TB 0.

3.4. Germination and Growth of Tomato Plants on Peat Substrate Mixed with Washed and Unwashed Biochar

Without water rinsing, only 33 ± 27% (4 of 10) of the seeds planted on the TB 0/peat substrate germinated after 28 DAS. This is in line with the observations by Nocentini et al. [3]. Washing two times considerably increased the rate to 75 ± 17%, which was already reached after 18 DAS (Figure 3, Table 5). Bearing in mind that washing had no impact on the pH values of the substrates but decreased their EC values (Table 4) considerably, the improved germination may be best explained by the removal of salts, which were toxic at levels occurring in the original TB. Salt stress reduces a seed's ability to absorb water (osmotic stress) and causes an ion imbalance within the seed (ionic stress), ultimately inhibiting germination and preventing crop production [8,26,35]. Such compounds could be cations, such as K, Na and Ca.

VB 0 amendment achieved 100% germination but only after 28 DAS. Respectively, after rinsing VB two times, the delay until maximal germination shortened to 12 days, which may be related to the decrease in the pH value. The better performance of the seeds germinating in VB-based substrates relative to those developing in TB-containing pots can be related to the comparably low EC of the former. It seems that in these substrate mixes, salt concentrations were still tolerated even at levels observed for VB0 [35]. Other phytotoxic compounds such as PAHs that could hinder germination may naturally be present in biochars from vines [36–38]. However, in our study, VB achieves 100% of total

germination even without washing, which indicates that in the VB-biochar used, such problematic compounds played a minor role, if any.

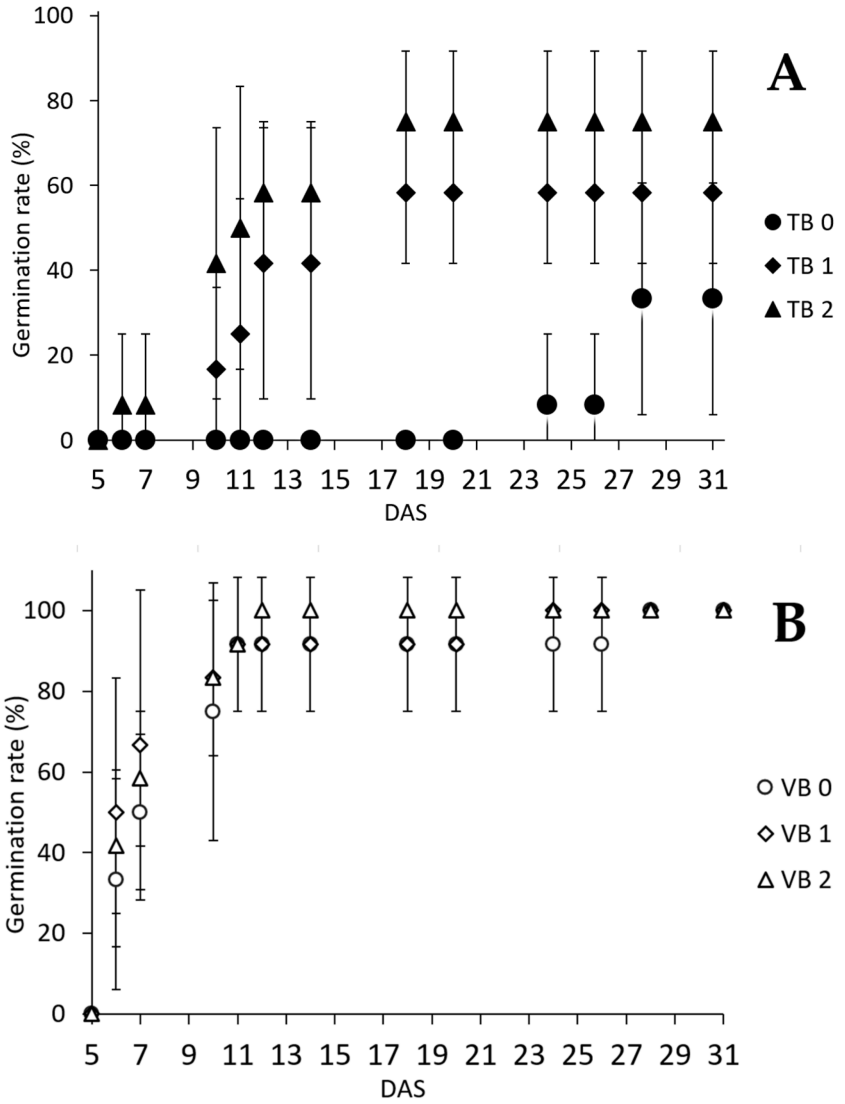


Figure 3. Accumulated percentage of germinated tomato seeds sowed on peat substrates mixed with biochar from (**A**) tomato greens (TB) and (**B**) vineyard pruning (VB) counted at days after sowing (DAS). TB 0 and VB 0 refer to the substrates with unwashed biochar; TB 1 and VB 1, and TB 2 and VB 2 refer to the biochars after one and two washings, respectively.

Table 5. Total accumulated germination (TG), plant height (PlantH), shoot weight (fresh weight: FW and dry weight: DW), plant water content (WC), leaf area (LA), chlorophyll indicator (SPAD), root dry weight (DW) and root-to-shoot ratio (R/S) of tomato plants cultivated under different peat:biochar substrates based on once-, twice- and non-washed biochars from tomato greens (TB 0, TB 1, TB 2) and vineyard pruning (VB 0, VB 1, VB 2). All values were determined at the end of the pot experiment (31 DAS).

Treatments	TG (%)	PlantH (mm)	Water Consumption (mL)	Shoot FW (mg)	Shoot DW (mg)	Plant WC (%)	LA (cm^2)	SPAD Index	Root DW (mg)	Dry R/S
TB 0	33 ± 27 b	4 ± 5 c	73 ± 1 a	4 ± 4 b	1 ± 1 b	81.6 ± 8.4 b	BD **	BD **	1 ± 1 b	1.3
TB 1	59 ± 17 ab	24 ± 6 b	64 ± 2 b	88 ± 21 b	7 ± 2 b	92.0 ± 0.5 a	2 ± 1 b	27 ± 6 b	2 ± 1 b	0.3
TB 2	75 ± 17 a	37 ± 7 a	79 ± 6 a	531 ± 171 a	51 ± 17 a	90.5 ± 0.5 a	16 ± 8 a	39 ± 2 a	22 ± 13 a	0.4
p	ns	***	**	***	***	*	**	***	**	-

Table 5. *Cont.*

Treatments	TG (%)	PlantH (mm)	Water Consumption (mL)	Shoot FW (mg)	Shoot DW (mg)	Plant WC (%)	LA (cm^2)	SPAD Index	Root DW (mg)	Dry R/S
VB 0	100 ± 0 a	35 ± 5 a	85 ± 10 b	446 ± 43 a	40 ± 4 b	91.1 ± 0.2 a	10 ± 2 a	31 ± 4 a	8 ± 5 c	0.2
VB 1	100 ± 0 a	45 ± 3 a	104 ± 5 a	438 ± 55 a	51 ± 6 b	88.3 ± 0.4 b	11 ± 2 a	29 ± 4 a	18 ± 3 b	0.4
VB 2	100 ± 0 a	42 ± 6 a	97 ± 11 ab	533 ± 96 a	80 ± 3 a	85.0 ± 0.6 c	14 ± 6 a	35 ± 5 a	28 ± 4 a	0.3
p	ns *	ns	*	ns	***	***	**	***	***	-
M	***	***	***	***	***	ns	**	***	**	-
W	*	***	*	***	***	*	***	***	***	-
M × W	*	**	**	***	ns	***	*	***	ns	-

* ns: no significant differences. ** BD: below detection. Values followed by different letters in the same column indicate significant differences according to Tukey's test. Levels of significance: "ns" $p > 0.05$. * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$. Statistical differences between material (M), number of washings (W) and their interaction (M × W) are shown at the bottom of the table.

One time washing of TB already increased plant growth with respect to those cultivated on the TB 0 mixture (Figure 4). A plant height of 37 ± 7 mm comparable with those obtained from unwashed and washed VB-containing substrates was only achieved with TB 2 (Figure 5, Table 5). These results indicate that at least two washings were required to sufficiently reduce the concentration of stress-inducing compounds in TB, thereby enabling plant growth comparable to that observed in the VB treatments. This occurred despite the delayed germination observed in the TB-based substrates.



Figure 4. Plant development at 31 days after sowing (DAS) on peat substrates mixed with unwashed, once- and twice-washed biochar (60:40 *v*:*v*) from vineyard pruning (VB 0, VB 1, VB 2) and tomato greens (TB 0, TB 1, TB 2). Starting from the left (in columns): VB 2, VB 1, VB 0, TB 2, TB 1 and TB 0.

Although plant weights were increased by biochar washing, the total water consumption of the plants growing in TB-containing substrates remained unaffected (Table 5). It varied between 64 ± 2 g for TB 1, and 79 ± 6 g for TB 2. For the VB treatments, on the other hand, a slight but not significant increase from 85 ± 10 g for VB 0 to 97 ± 11 g for VB 2 was revealed. These results suggest that evaporation likely accounted for a major proportion of the water loss, although the relative contribution of plant transpiration cannot be excluded. It may be further concluded that the salt content of the substrate had no major impact on its water retention capacity. It has been reported that washing can increase the specific surface area and total pore volume of biochar by removing soluble components that could block the pores [39]. However, there were significant differences between the two types of

biochar, with greater water consumption for VB treatments than for TB treatments either due to different water loss from evaporation or, what is more likely, the better plant growth on VB [1].

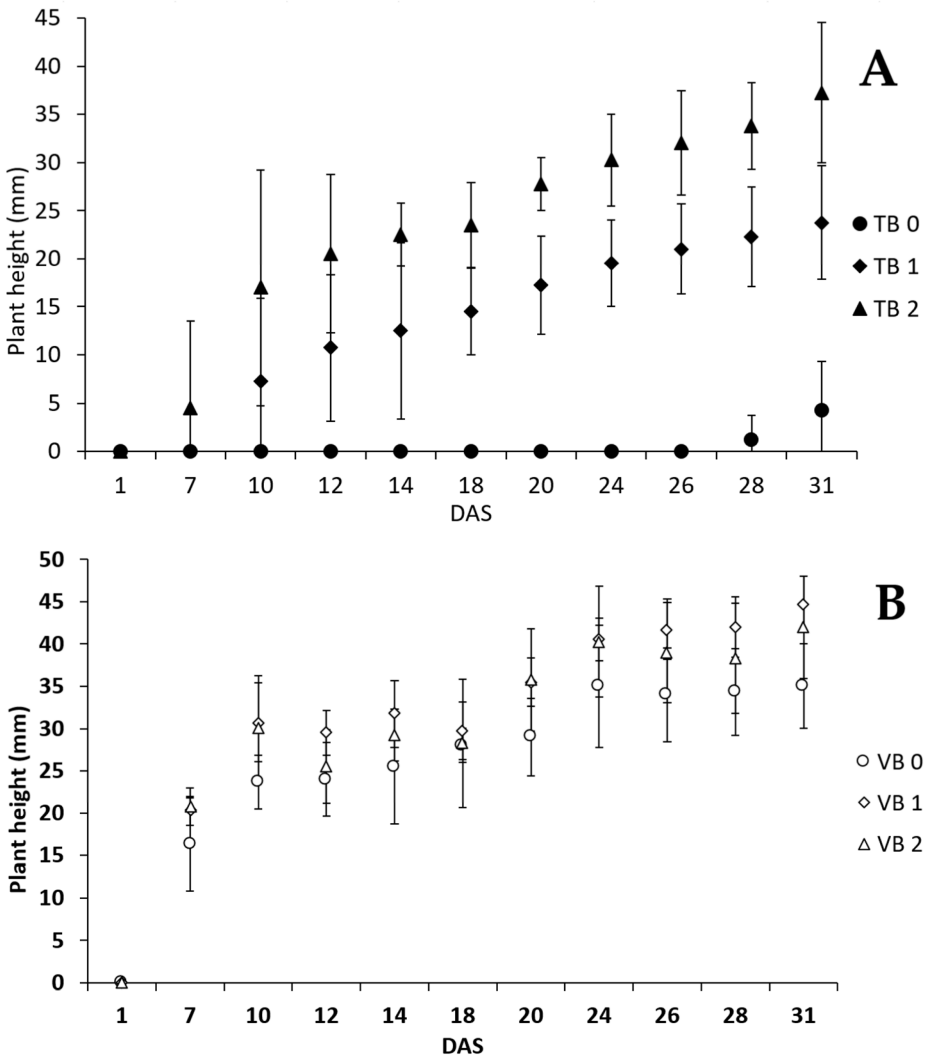


Figure 5. Evolution of plant height of tomato plants cultivated on biochar:peat substrate with days after sawing (DAS) with (**A**) unwashed (TB 0), once- (TB 1) and twice-washed (TB 2) biochars from tomato greens and (**B**) from vineyard pruning (VB 0, VB 1, VB 2), respectively.

The shoot fresh and dry weight of the plants showed the same trend with biochar treatment as the plant height. However, while the water content of the plants increased after biochar washing for TB-containing substrates, it decreased for the VB-based experiments. At the same time, the leaf area increased for the plants on both biochar substrates with washing. This leads to the assumption that washing of the biochar before their amendment enhanced succulence for plants in TB substrates but decreased it for those in VB-based pots. On the other hand, dry mass weight of shoots increased for both biochars with washing, which is in line with results reported by Intani et al. [8] and Rogovska et al. [37].

With the exception of the TB 0 treatment, the dry root-to-shoot ratio ranged between 0.2 and 0.4, which is slightly higher than the values reported by Naciri et al. [39] for tomato plants grown in hydroponic systems (0.1–0.2). Elevated root-to-shoot ratios are commonly associated with environmental stress, as plants allocate proportionally more resources to root development to improve resource acquisition. This pattern was particularly evident in plants cultivated in the TB0 substrate, which exhibited markedly higher root-to-shoot

ratios [40]. SPAD values are used as indicators of chlorophyll contents and therefore provide an additional index for plant health [5,21]. Whereas for TB 0 no values could be obtained due to low leaf production, TB 2 (39 ± 2) provided the best value. For the VB treatments, no significant impact of biochar washing on the SPAD was revealed [8,35].

3.5. Pearson Correlation: Revealing the Relations Between Micronutrients and Other Parameters

Summarizing the results of the plant physiological analysis, the VB and TB treatments had a considerable different impact on plant health which cannot be explained with high alkalinity only. In order to obtain a better understanding of which parameter was responsible for the low performance of TB 0, a Pearson correlation was applied (Figure 6).

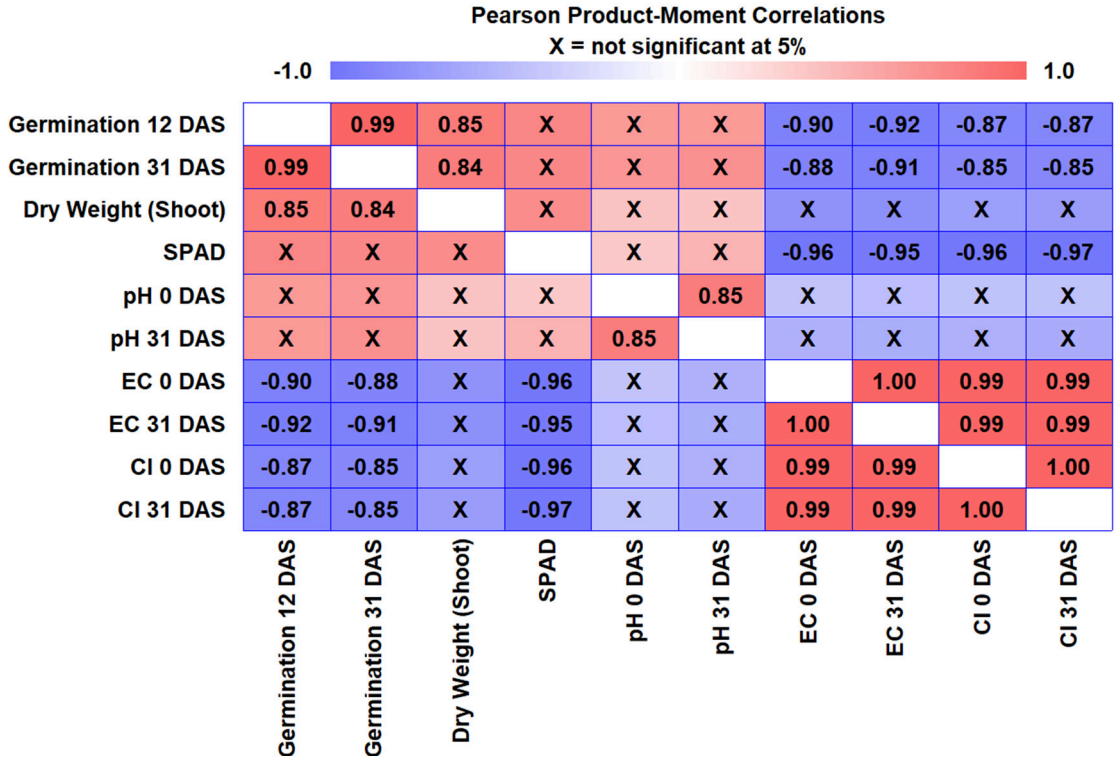


Figure 6. Pearson product-moment correlation matrix. Parameters: Germination (at 12 and 31 DAS), shoot dry weight at 31 DAS, SPAD, pH (at 0 and 31 DAS), EC at 0 and 31 DAS, and Cl^- content at 0 and 31 DAS. Correlations: Not significant at 5% (X), positive and significant at 5% (red); negative and significant at 5% (blue).

Correlating substrate pH with germination and plant performance parameters indicated a minor impact, likely because even after two washings, the pH remained above the optimal range for tomato plants [13]. In contrast to pH, changes in EC exerted a clear negative impact on germination, which was statistically significant at the 5% level. This is best explained by osmotic stress. Following germination, a strong negative correlation between EC and plant performance persisted, though this relationship was only significant at the 5% level for the SPAD value. Potentially, in addition to osmotic stress, nutrient antagonism emerged as a critical factor influencing plant development. Relating the concentrations of Na, Cl, Ca, K, and S in the biochar to germination revealed statistically significant correlations ($p < 0.05$) (Figure 7). Although Na and Cl contents affected shoot dry weight ($r = -0.73$ and $r = -0.70$, respectively), only Ca and K had a statistically significant impact on this parameter. This trend further points toward nutrient antagonism, as excessive Ca and K can promote antagonism and inhibit root development or chlorophyll synthesis [41,42]. The impact of K on chlorophyll production was supported by a strong,

statistically significant negative correlation between K content and SPAD. However, significant negative correlations were also observed between SPAD and Na, Cl, and S. In contrast, the P content of the biochars showed no major impact on plant health parameters.

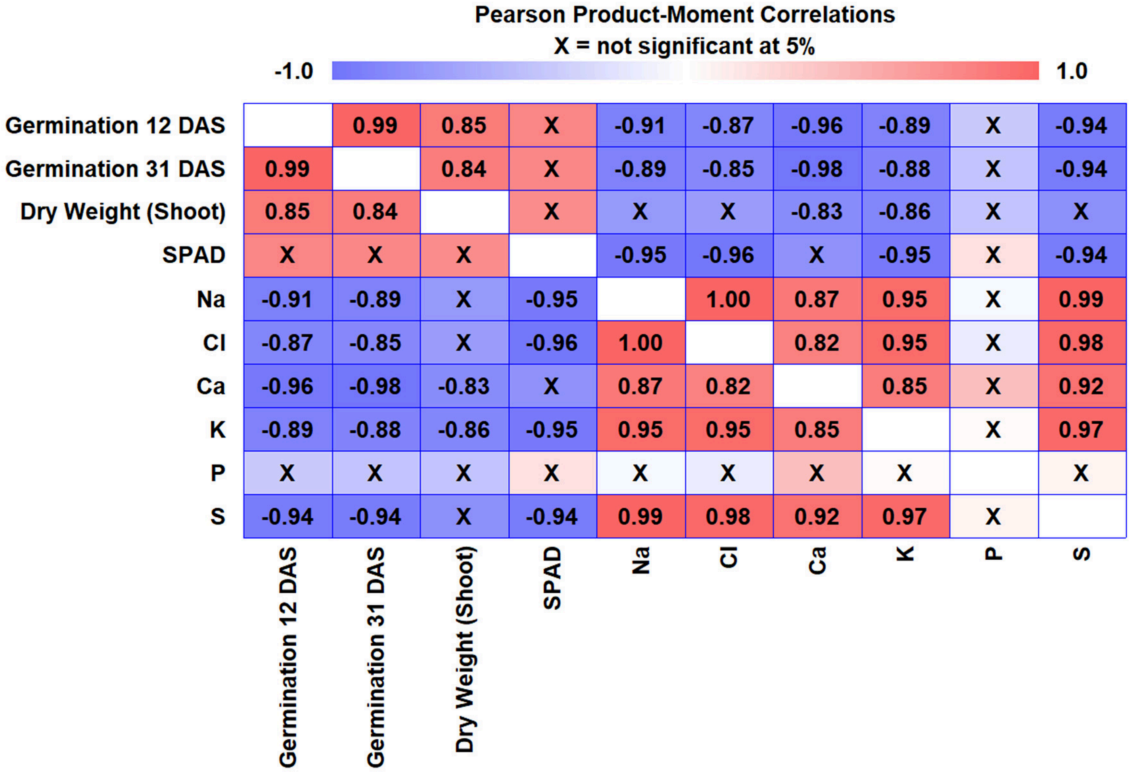


Figure 7. Pearson product-moment correlation matrix. Parameters: Germination (at 12 and 31 DAS), shoot dry weight production at 31 DAS, SPAD and ions contents (Na, Cl, Ca, K, P, S) of the biochars. Correlations: Not significant at 5% (X), positive and significant at 5% (red); negative and significant at 5% (blue).

3.6. Principal Component Analyses: Revealing the Influence of Biochar Washing on Their Parameters

Principal component analysis (PCA) was performed to identify the biochar parameters that most strongly influenced tomato plant performance. The biplot (Figure 8) shows that PC1 explains 68.37% and PC2 11.91% of the total variance, together accounting for approximately 80% of the variability, indicating a robust representation of the dataset.

PC1 clearly separates the treatments along a gradient from stress-related conditions to plant growth performance. On the negative side of PC1, TB 0 is associated with high EC and Cl^-, indicating saline stress. In contrast, all VB treatments as well as TB 2 are located on the positive side of PC1 and correlate with growth-related traits such as shoot FW, shoot DW, LA, root DW (RW), total germination (TG), plant height (PlantH), and SPAD values.

The shift from TB 0 to TB 1 and TB 2 along PC1 reflects the effect of washing, which reduced EC and Cl^- and thereby alleviated plant stress. Notably, TB 2 clusters with the VB treatments on the positive side of PC1, indicating improved growth conditions. All VB treatments are located on the positive side of PC1 and show comparatively smaller separation along PC1 than the TB treatments. This indicates that variation in salinity had a less pronounced effect within the VB treatments, possibly because in none of the treatments, salt levels were extensively over the phytotoxic threshold.

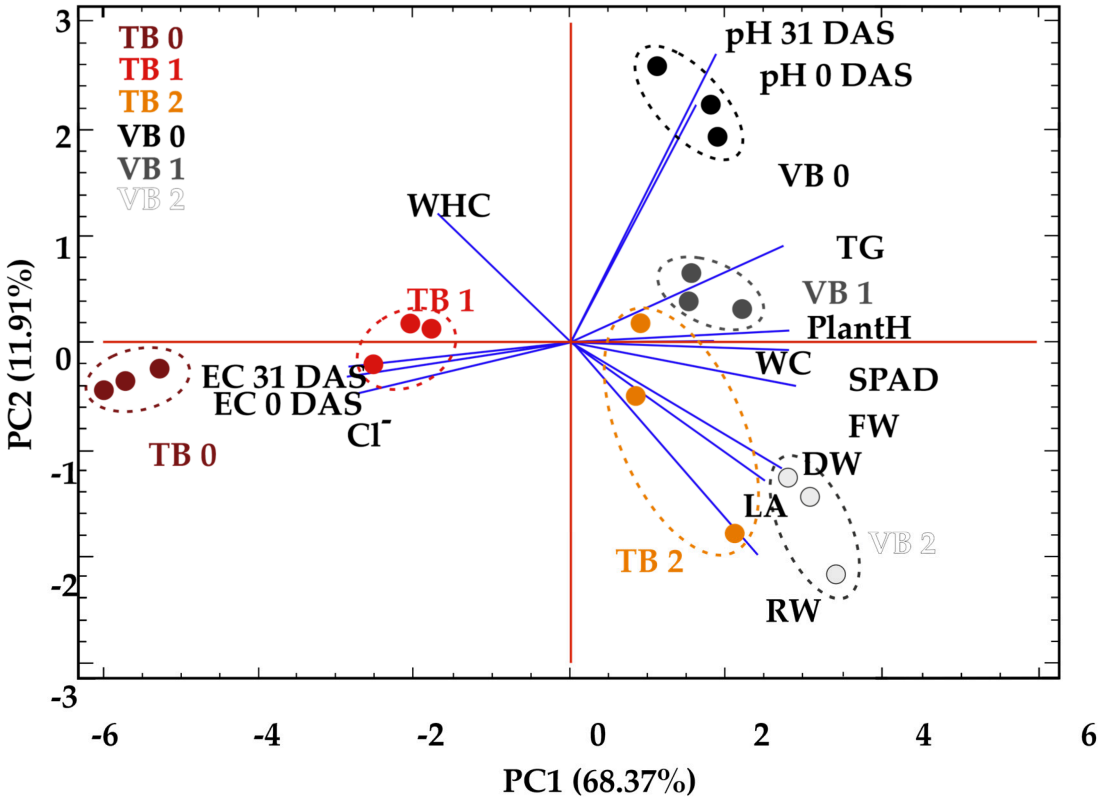


Figure 8. PCA biplot with the first two PCA axes, with projected centroids of treatments based on tomato biochar (TB 0-dark red, TB 1-red and TB 2-orange) and vineyard biochar (VB 0-black, VB 1-gray and VB 2-white). Substrate parameters (pH (at 0 and 31 DAS), EC (at 0 and 31 DAS), Cl^- content (Cl^-), water consumption (WC) and WHC) and plant parameters (total germination (TG), plant height (PlantH), shoot fresh weight (FW), shoot dry weight (DW), root weight (RW), leaf area (LA) and SPAD) are shown.

PC2 is largely driven by variation in substrate pH. The vectors of pH at 0 and 31 DAS are directed towards the upper right quadrant. The directions of the vectors of total germination (TG), plant height (PlantH) and SPAD are more affected by PC1 than by PC2, which may indicate the stronger impact of EC than of pH on those parameters. The other plant growth parameters (FW, DW, LA, RW) are oriented towards the lower right quadrant. VB 0 is located in the upper right region, suggesting an association with higher pH, while VB 2 is situated in the lower right quadrant and is closely aligned with biomass-related traits, suggesting a strong association with enhanced plant performance.

Overall, the PCA indicates that in our experiments, salinity-related parameters (EC and Cl^-) have a stronger influence on plant performance than substrate pH. However, here it has to be borne in mind that in none of our trials the optimal pH range for tomato plant growth was achieved.

To further elucidate the chemical drivers underlying the observed growth responses, a second PCA was performed using plant growth parameters and individual ion concentrations in the biochars (Figure 9). This analysis allowed the identification of ions most strongly associated with plant performance and helped distinguish their relative contributions from the overall salinity effect detected in the first PCA (Figure 8).

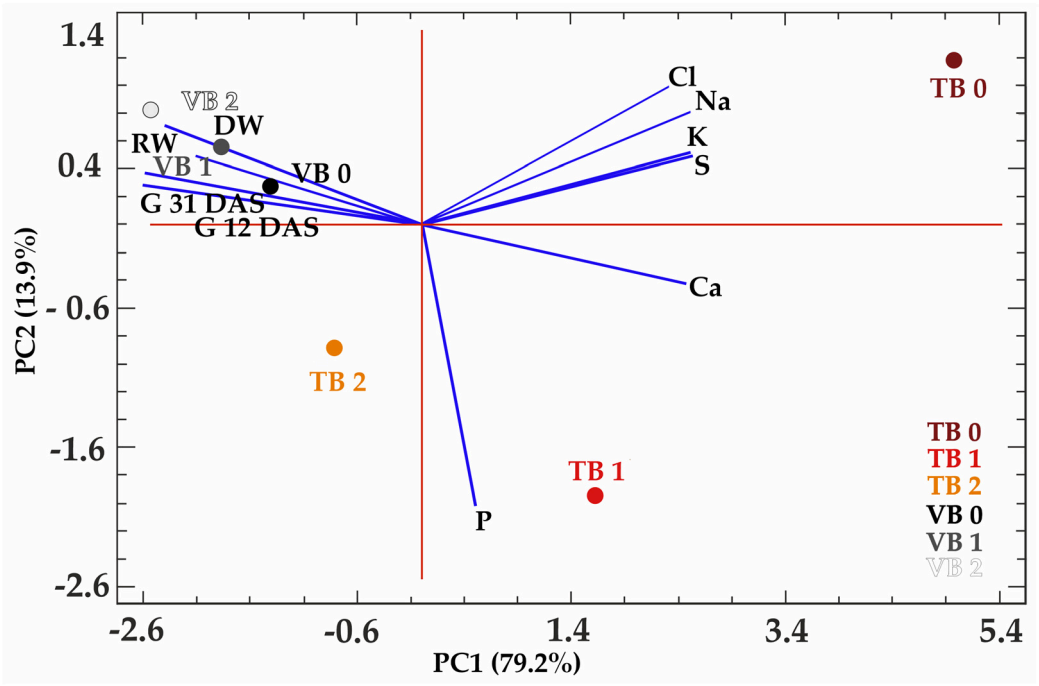


Figure 9. PCA biplot with the first two PCA axes, with projected centroids of treatments based on tomato greens biochar (TB 0-dark red, TB 1-red and TB 2-orange) and vineyard pruning biochar (VB 0-black, VB 1-gray and VB 2-white). Shown plant parameters: Germination at 12 DAS (G 12 DAS), germination at 31 DAS (G 31 DAS), shoot dry weight (DW), root dry weight (RW), and content of biochar ions (Na, Cl, Ca, K, P, S).

The respective biplot in Figure 9 shows that PC1 explains 79.2% and PC2 13.9% of the total variance, together accounting for approximately 93% of the variability, indicating a robust representation of the dataset. PC1 clearly separates treatments according to their association with plant performance and ion concentrations in the biochars. The strong separation along PC1, which accounts for nearly 80% of the total variance, indicates that differences in ion composition and their association with plant performance represent the dominant source of variation among treatments.

Here, the positive side of PC1 is associated with elevated concentrations of Na, Cl, K and S which are negatively correlated with germination and growth performance and may reflect stress-related conditions. The close grouping of the germination and biomass vectors indicates that these growth parameters responded similarly across treatments. Likewise, the clustering of Na, Cl, K and S suggests that these ions co-varied and collectively contributed to treatment differentiation. The distribution of the VB and TB treatments in the PCA space indicate that the VB was generally more favorable than TB for plant development. In contrast to TB treatments, VB 0, VB 1 and VB 2 are all clustered relatively closely and along vectors assigned to plant performance, suggesting that washing had comparatively modest effects on the position of VB treatments in the PCA space. TB 0 and TB 1 are strongly associated with Na, Cl, K and S, suggesting that these ions are important drivers of the differences among TB treatments. TB2 occupies an intermediate position, being located on the negative side of PC1 but clearly separated from the vineyard biochar treatments along PC2. This indicates that washing substantially improved the suitability of TB as a substrate additive, although its overall chemical characteristics remained distinct from those of VB. P is primarily associated with PC2, suggesting that this factor contributes to treatment differentiation through a different mechanism. The distinct orientation of the P vector indicates that P varies independently from Na, Cl, K and S. Unlike these ions, P is not aligned with the negative growth response pattern represented by PC1.

The behavior of Ca is different from both the salinity-related ions (Na, Cl, K, S) and P. Although Ca loads positively on PC1 and therefore contributes to the separation between VB and TB treatments, it differs from Na, Cl, K and S by loading negatively on PC2. This suggests that Ca varies independently from these ions and is not aligned with negative the growth-response pattern observed for Na, Cl, K and S.

Overall, the PCA supports the conclusion that elevated concentrations of Na, Cl, K and S are the principal chemical factors associated with reduced tomato germination and growth, whereas the vineyard biochar treatments are associated with more favorable growth conditions.

4. Conclusions

The results of this study demonstrate that washing with water is an effective method for improving the performance of biochars as plant-growing substrates, particularly those initially characterized by high salinity and alkalinity. Our findings show that successive washing treatments reduce pH and substantially lower EC through the removal of soluble salts and alkaline compounds. The removal of these components from the tested biochars produced a substrate additive that supported tomato seed germination and subsequent plant growth even at high application rates of 60% (v/v).

Importantly, the objective of this study was not to identify an optimal horticultural substrate formulation, but rather to elucidate the mechanisms by which washing alters biochar properties and influences plant performance. In this regard, the results clearly demonstrate that reductions in salinity-related parameters are more closely associated with improvements in germination and growth than changes in pH. Indeed, pH and EC showed no clear correlation, indicating that pH was not solely governed by the concentration of soluble salts. Together with the ^{15}N NMR evidence for aromatic nitrogen-containing domains in TB, this observation suggests that pH buffering by heterocyclic nitrogen structures may have contributed to the observed pH behavior.

Overall, our study highlights that biochars with high salt content and elevated pH—previously considered "virtually unusable" as peat substitutes in horticultural soils due to their adverse properties—can be transformed into valuable substrate additives through a simple washing treatment. Our findings further support the view that biochar should not be regarded as a homogeneous material class with uniform effects on plant health and soil properties, but rather as a product whose characteristics are strongly determined by its feedstock. As demonstrated in the present study, feedstock influences not only the properties of the organic aromatic network and porosity, but also the composition of the inorganic fraction. In particular, managing salinity-related ions through feedstock selection or post-treatment processes such as washing is critical for optimizing biochar performance in plant production systems. From a circular economy perspective, however, the water consumption associated with this treatment must also be considered. One potential strategy is the reuse of process water for irrigation, provided that its salt concentration remains sufficiently low to avoid soil salinization. Future studies should therefore evaluate both the agronomic and environmental implications of biochar washing, including opportunities for water recycling and resource-efficient process design.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/environments13070371/s1, Table S1: Contents of metals in the fresh biochars of tomato greens and vineyard pruning (TB 0 and VB 0), the respective biochars after the first (TB 1 and VB 1) and second washing (TB 2 and VB 2), and the peat; Table S2: Intensity distribution in the solid-state ^{13}C NMR spectra of the substrates with the fresh biochars from tomato greens and vineyard prunings (TB 0 and VB 0), and the respective biochars after the first (TB 1 and VB 1) and second washing (TB 2 and VB 2) before (0 DAS) and after incubation (31 DAS).

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