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Composting a Mixture of Cactus Pear Pruning Waste and Spent Coffee Grounds: The Chemical Evaluation of Organic Fertilizer in Response to Basil Quality and Growth

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Abstract: In specialized orchards, approximately 6–10 tons/hectare of cactus pear pruning waste and 60 million tons of spent coffee grounds are estimated to be produced each year worldwide. Composting is a process that produces stable organic matter useful in agriculture. The aim of this work was to explore the potential of *Opuntia ficus-indica* (OFI) cladodes and spent coffee ground (SCG) mixtures for compost production and to assess their benefits for agricultural applications. Three composting campaigns were carried out using rotating composters. Feedstock for these campaigns was formulated with different ratios of OFI and SCGs, and the compost obtained were characterized by their chemical and physical proprieties. To assess these composts, basil was grown in plots using growing substrate as a blank and comparing it with substrate mixed with 10% of each compost. All plants sprouted and grew up. While no significant differences were detected in polyphenol content among the grown plants, the yields with compost at OFI–SCG (3.3:1) were differentiated for longer shoots and there was greater biomass compared to the control. Compost obtained from cladode mixed with spent coffee grounds proved to be a good soil improver with the characteristics of being able to ameliorate soil fertility and plant growth.

Keywords: *Opuntia ficus-indica*; spent coffee grounds; composting; waste valorization; soil fertility; basil; plant basil growth; polyphenols; antioxidant activity

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1. Introduction

Opuntia ficus-indica ((L.) Mill.), commonly known as the cactus pear, is a succulent plant that has adapted to extreme climatic conditions. To cope with water scarcity and high temperatures, this plant has evolved cladodes, a morphological change in branches with photosynthetic ability, which are covered in thorns that are essential to reduce excessive perspiration. Cladodes' main constituents are water, fibers, polysaccharides, proteins, fatty acids, vitamins, sterols, minerals and polyphenols [1–3].

Commercial applications of OFI require extensive cladode pruning, thus producing significant amounts of byproduct [4]. These discarded cladodes are typically not reused,

becoming waste and a burden to the value chain. However, cladodes exhibit a high concentration of an extractable polysaccharide named mucilage that can be used in many applications. This mucilage has been found to have a high concentration of calcium and potassium, along with a fair number of health-promoting phytochemicals, thus representing an excellent source of nutrient supplementation [1,5,6]. Mucilage from OFI cladodes has also revealed useful characteristics, such as bio-mortars for restoration applications [7]. Moreover, the antioxidant, anti-inflammatory and anti-apoptotic properties have opened new research avenues into the potential pharmaceutical applications of these cladodes [8,9]. Recently, the potential of OFI cladodes as a carbon source for biohydrogen and for volatile fatty acid production via mesophilic dark fermentation was investigated [10].

Another promising application of OFI properties is for use as a bio-fertilizer capable of combating desertification. In fact, by treating OFI's byproducts through the aerobic processes of composting, a soil improver with very high water retention and nutrient availability can be produced [11]. However, further research is needed to fully understand the implications of using cladodes in composting and their effects on soil and plant health.

Coffee consumption is a daily ritual for millions around the globe, leading to the production of a significant amount of organic waste in the form of spent coffee grounds. SCGs contain large amounts of organic compounds (i.e., fatty acids, amino acids, polyphenols, minerals and polysaccharides) that have huge potential for valorization [12–14].

Traditionally viewed as waste, SCGs emerged as a valuable resource for different applications, such as biofuel, energy, biopolymer precursors and composite production [15–17]. Additionally, the valorization of SCGs in agriculture by means of composting represents a sustainable practice to obtain a high-quality soil amendment thanks to their high amount of organic matter and nutrients, and to the low risk of heavy metal contamination that ensures a final product with no contaminants. The utilization of SCGs in composting thus provides a sustainable alternative to synthetic fertilizers in the context of circular economy [18–20].

By valorizing these two byproducts and preventing them from becoming waste, an interesting opportunity to produce an excellent organic fertilizer opens up. To increase the concentration of macro- and micronutrients in a green waste compost, such as that produced with OFI byproducts which are typically low in these components, several studies have used co-substrates to improve product quality and/or to reduce the green waste composting process duration [21–23]. SCGs are regarded as an important source of nitrogen (N) to reduce N gaseous loss and increase the N content in the compost product [24–26].

The aim of this work was to explore the potential of OFI cladodes and SCG mixtures for the composting process by examining the final product composition, its quality and its implications for agricultural applications. We hope that our findings will contribute to the sustainable management of OFI pruning and SCG recycling and highlight the untapped potential of these underutilized resources.

2. Materials and Methods

2.1. Composting Campaigns

Three composting campaigns were carried out using domestic rotating composters. The feedstock's tested compositions varied according to the ratios of OFI and SCGs presented in Table 1. These mixtures were formulated to obtain a ratio of OFI–SCG of 10:1, 3.3:1 and 2:1 which were named, respectively, as C1, C2 and C3. An additional feedstock of pure OFI (C0) was composted as a reference. In our previous work [11], we demonstrated that raw cladodes are an excellent substrate for aerobic fermentation, providing a final product of good quality, with the characteristics of a buffer capacity during the active phase of the process, a high moisture content at the end of the curing and a high potassium content. The

addition of spent coffee grounds in the mixture in smaller quantities, up to a maximum of 50% by weight of the *Opuntia* (OFI–SCG—2:1), was hypothesized to provide nitrogen to the final soil improver, since the content of this element was low in the compost obtained from *Opuntia* alone.

Table 1. Composition of feedstocks used for composting campaigns.

Material (kg)/Sample	C0 Only OFI	C1 OFI–SCG 10:1	C2 OFI–SCG 3.3:1	C3 OFI–SCG 2:1
Opuntia ficus-indica (OFI) cladodes	61	56	47	41
Spent coffee grounds (SCGs)	0	6	14	20
Bulking agent (BA)	13 *	13 *	11	11
Inoculum (cured compost)	1	1	1	1
Tot. (kg)	75	75	73	73

* For the C0 and C1 trials, 2 kg of bulking agents was added during the 6th week of the experiment to increase the porosity.

Each composter was fed once a week for 5 weeks with 14 kg of organic matter, of which 2 kg was represented by a bulking agent (BA) and the remaining by the OFI–SCG mixtures. Each substrate mixture was prepared by individually weighing the OFI, SCG and BA; manually mixing the materials in a tray and then loading the mixture into the composters. To facilitate the start-up process, an equal amount of inoculum (1 kg of cured compost) was added to each of the composters. The main characteristics of cured compost used as inoculum are reported in the Supplementary Materials (Table S1).

Arundo-donax cane prunings were used as a bulking agent. These reeds were chopped to a size roughly between 2 and 5 cm. OFI high-water-content sawdust was used instead of chopped reeds for the last feed to limit leachate production. Moreover, during the 6th week of operation, for C0 and C1, a visual examination revealed a lack of porosity, and to correct this, it was decided to add an additional 2 kg of chopped reeds. It was speculated that the higher quantity of SCGs added for the other two tests, C2 and C3, thanks to their fine granularity, acted as an anticaking agent, thus eliminating the need for an additional bulking agent.

After the initial feeding weeks, the composting trials were kept in the rotating composter for an additional 23 weeks from November 2021–March 2022. Subsequently, the produced composts concluded the curing in heaps from April 2022 to August 2022 for a total duration of 9 months.

The total quantities loaded for each composter are shown in Table 1.

The temperature within each composter was logged by means of a near-real-time monitoring system realized by ENEA using an Internet of Things solution. The monitoring system was based on a microcontroller ESP32 board (Xinyuan-LilyGo-T-Call-SIM800 Series) with a SIM800L GSM/GPRS module for internet connection. A DS18B20 temperature sensor (digital temperature sensor with ± 0.5 °C accuracy from -10 to +85 °C) was connected to the ESP32 board and, through the SIM800L modem, the readings were sent every 30 min to an online database and visualized on a dashboard realized with Looker Studio, an online tool for converting data into informative dashboards (Google LLC, Mountain View, CA, USA).

Conversely, once the material was moved from the rotating composters into heaps, the temperature was monitored once a week, on average, by a long rod thermometer (TC Direct).

Water content was determined by drying at a constant temperature of 105 °C with a thermobalance (Crystal Therm, Gibertinim, Novate Milanese, Italy) and pH was monitored

by a portable meter (HI99121, Hanna Instruments, Villafranca Padovana, Italy) designed specifically for soil analysis.

The ultimate analyses of raw products (OFI and SCGs) and composts at the end of the curing phase were performed to determine the total carbon and total nitrogen content by the elemental analyzer (Elementar, vario MACRO, Lomazzo, Italy). Trace metal (Cd, Cu, Fe, Mn, Ni, Pb and Zn) content determinations in raw products (OFI and SCGs) were performed by ICP-MS (using an ICP-MS Agilent 7800) and major element (Ca, Mg, K and P) concentrations were determined by ICP-OES (with ICP-OES-Perkin Elmer-Optima 200DV, Springfield, IL, USA). Before carrying out the analyses, each sample was solubilized using a microwave-assisted acid dissolution procedure. The determinations of Cr (VI) were carried out on the water-leachable chromium fraction; the aqueous extracts of each sample were then analyzed by ICP-MS. The content of mercury (Hg) was measured directly in the samples, without any further treatment, using an AMA—254 (FKV, Milestone, Sorisole, Italy) spectrometer. All the results were expressed as a % of the dried weight [27,28].

2.2. Basil Plant Growth Experimental Trial

To evaluate whether the integration of OFI–SCG composts into the soil was a suitable option for plant growth, an experiment using basil plants in pots (20 cm diameter) was carried out. Commercial potting soil, commonly used for nurseries (TerComposti SpA; Calvisano, Italy; main characteristics: pH (in H_2O) = 8; electrical conductivity = 0.8 dS m^{-1}; dry bulk density = 220 kg mc^{-3}; total porosity = 82%), was utilized to prepare five soil samples, as reported in Table 2, for a total of 45 samples (3 plants/plot and 3 plots for treatment) placed in three randomized blocks.

Table 2. Description of soil samples used for experimental trial on basil plant growth.

Soil Samples	Description
Ctrl	Control, only commercial soil
Ctrl + 10% C0	Commercial soil supplemented by 10% of C0 compost (OFI only)
Ctrl + 10% C1	Commercial soil supplemented by 10% of C1 compost (OFI–SCG 10:1)
Ctrl + 10% C2	Commercial soil supplemented by 10% C2 compost (OFI–SCG 3.3:1)
Ctrl + 10% C3	Commercial soil supplemented by 10% of C3 compost (OFI–SCG 2:1)

Seeds of basil (*Ocimum basilicum* (L.), *Genovese* type) purchased from Agrarian Consortium Dorelli (Rome, Italy) were surface-sterilized with 5% sodium hypochlorite solution for 20 min, washed four times with sterile water and allowed to germinate in the pots. Seedlings were grown in a climatic chamber at 24 ± 2 °C with a photoperiod of 16 h light/8 h dark and watered weekly. Ten leaves per plot were collected at the basal part of the plant after 1, 2 and 3 months from sowing (T1, T2 and T3). The leaves were immediately weighed (FW, g), while the dry leaf weight (DW, g) was evaluated in leaves maintained in a forced-air oven at 60 °C for 3 days until reaching a constant weight. The percentage of leaf water content (WC%) was calculated as $WC = [(100 \times (FW - DW) \times FW^{-1})]$ and dry matter leaf percentage (DM%) was calculated as $DM = 100 \times DW/DF$. Four months after sowing, the plants were carefully explanted, and the roots were gently washed with running tap water to remove soil residues. After shoot separation from roots, the following characteristics were evaluated: fresh weight of root biomass (g); root length (cm); fresh mass of the aerial part (g); plant height (cm) and number of nodes with expanded leaves in the main stem. All samples were then lyophilized using a bench freeze-dryer (LIO-SPDGT, 5Pascal) and weighed.

2.3. Polyphenol Extraction

For chemical analysis, 400 mg of lyophilized leaves were extracted, with 20 mL of 70% EtOH as an extractive solvent, for 30 min in a sonic bath (frequency: 49 KHz; 120 W; 25 °C). The extracts were then centrifuged for 10 min at 16.500× *g*, filtered with a 0.22 µm cellulose acetate filter and stored at −20 °C [5].

2.4. Total Polyphenol Content

The Folin–Ciocalteu [29] method with slight modifications was used to quantify the total polyphenol content. Briefly, aliquots of 200 µL of each extract were mixed with 1.5 mL of 0.2 N Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MI, USA). A blank sample was prepared by adding 200 µL of 70% EtOH. Each sample was then stirred in a bench Vortex mixer and allowed to stand for 5 min in the dark. After this time, 1.5 mL of anhydrous sodium carbonate (60 g L^{-1}) (Sigma, St. Louis, MI, USA) was added to the reaction mixture. Finally, the samples were allowed to stand in the dark for about 30 min until the end of the reaction. The resulting samples' absorbance (Abs) was read at 765 nm using a UV/Visible spectrophotometer (Lambda 950—Perkin Elmer). All measurements were carried out in triplicate and the results were expressed as the amount of gallic acid (≥99% Sigma, St. Louis, MI, USA) in ethanol at four known concentrations (100, 250, 500, 750 mg L^{-1}).

2.5. Flavonoid Content

The reaction mixture was prepared by mixing 1.5 mL of 95% ethanol with 2.8 mL of ultrapure water. To the solvent thus prepared, 100 µL of potassium acetate 1 M and 100 µL of aluminum chloride 10% *w*/*v* were then added. At the end of the reaction, after incubation for 30 min in the dark, the sample absorbance was read at 415 nm using a UV/Visible spectrophotometer (Lambda 950—Perkin Elmer). To calculate the concentration of total flavonoids, a first order calibration curve (Abs = 0.0081C − 0.0321; r^2 0.999) was obtained using a series of ethanol solutions of quercetin (Sigma, St. Louis, MI, USA) as reference standards at different concentrations (10, 20, 40 and 80 µg mL^{-1}). The total flavonoid equivalent concentration was expressed as µg quercetin equivalents g^{-1} of dry matter weight (µgQE g^{-1} DW). All measurements were carried out in triplicate.

2.6. Total Antioxidant Activity

The total antioxidant activity was measured by the Blois (1958) method with modifications [11]. Briefly, 100 µL of extract was added to 2.9 mL of 60 µM DPPH solution (Sigma, St. Louis, MI, USA). The samples were shaken in a Vortex mixer and kept in the dark until the end of the reaction after 60 min. Absorbance was read at 517 nm using a UV/Visible spectrophotometer (Lambda 950—Perkin Elmer). The control sample was a DPPH 60 µM solution. A blank was prepared by adding 100 µL of 70% MeOH to 2.9 mL of DPPH 60 µM. The % inhibition of the radical DPPH was obtained by interpolation of the first order calibration curve (Abs = 0.3466 C + 2.0431; r^2 0.9999) obtained by plotting the absorbance as a function of the concentration of the reference standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma, St. Louis, MI, USA). A series of four standard solutions in a concentration range of 50, 100, 250 and 500 mM were prepared, for each of which the % inhibition was calculated. By interpolation of the calibration curve (A vs. µM Trolox) the % inhibition was calculated as follows:

$$\% \text{ inhibition} = \{[(A_c - A_s) - (A_c - A_b)]/A_c\} \times 100$$

A_c = Control absorbance (DPPH 60 µM);
As = Absorbance of the sample;
A_b = Absorbance of the blank.

Results were expressed as μmol of Trolox equivalent g^{-1} of dry matter (μmol TE g^{-1} DW). All measurements were carried out in triplicate.

2.7. Ferric Reducing Antioxidant Power (FRAP)

This test measures the reducing activity of the Fe^{3+}TPTZ complex (Sigma, St. Louis, MI, USA), which is directly proportional to the formation of the Fe^{2+}TPTZ complex by the action of reducing substances in the sample. For this purpose, a reaction mixture was prepared by mixing, with a volumetric ratio of 45:5:5, three solutions consisting, respectively, of acetate buffer 300 mM at pH 3.6 + TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) 10 mM + $FeCl_3$ 20 mM, and keeping the mixture incubating in the dark at 37 °C for 60 min, up to the formation of the aforementioned Fe^{3+}complex. At the end of this time interval, 100 μL of each sample extract was added to 8 mL of the above mixture. A blank was prepared by adding 100 μL of 70% MeOH to 8 mL of the reaction mixture. The samples were then allowed to incubate again in the dark at 37 °C for 60 min until the end of the reduction reaction. The absorbance of each sample was read at 593 nm in a Lambda 950 Perkin Elmer UV-Vis spectrophotometer. Measurement of the reducing activity of each sample was obtained by the interpolation of a first order calibration curve obtained by measuring the absorbance of a series of solutions with known concentrations of $FeSO_4$ (0.1, 0.4, 0.8, 1.0, 1.5 μM) (Sigma, St. Louis, MI, USA). This activity was expressed in mmol$FeSO_4$eq g^{-1} DW. All measurements were carried out in triplicate.

2.8. Statistical Elaborations

The data were expressed as mean ± standard deviation or the standard error of three independent experiments, with at least three technical replicates in each experiment. $p < 0.05$ was considered statistically significant. The significance of difference was calculated using one-way ANOVA and Tukey's Test as the post-test. Statistical analysis was performed using SPSS statistic software version 23.

3. Results

3.1. Composting Campaings

Temperature is an important parameter for assessing the composting process and microbial activity, mainly being responsible for the degradation of organic matter. The temperature profiles of each mixture, as well as the external air temperature where the composting campaigns were carried out, are illustrated in Figure 1. Monitored temperatures reached peak values of 16.2 °C, 31.0 °C and 48.7 °C for C1, C2 and C3, respectively. Compared to air temperature, mixtures with higher SGC percentages showed higher temperature differences. The highest peaks were obtained for each mixture during the first week, and significantly lower temperature peaks were visible after each weekly load of new substrate. The main drawback of using domestic composters is the poor thermal insulation that prevents the retention of the heat produced by the microorganism's activity, despite the fair amount of organic material being used for the tests.

Moisture content influences the physiological characteristics of microbes and the physical structure of solid matrices during composting. If moisture content is maintained at a proper level, aerobic microorganisms show more active oxygen consumption during composting due to the increased microbial activity.

pH value changes throughout the composting process due to changes in chemical compositions as materials decompose. Initially, pH will fall below neutral due to the formation of organic acids. Then, the pH will increase as microbes use these acids, and as proteins are being decomposed.

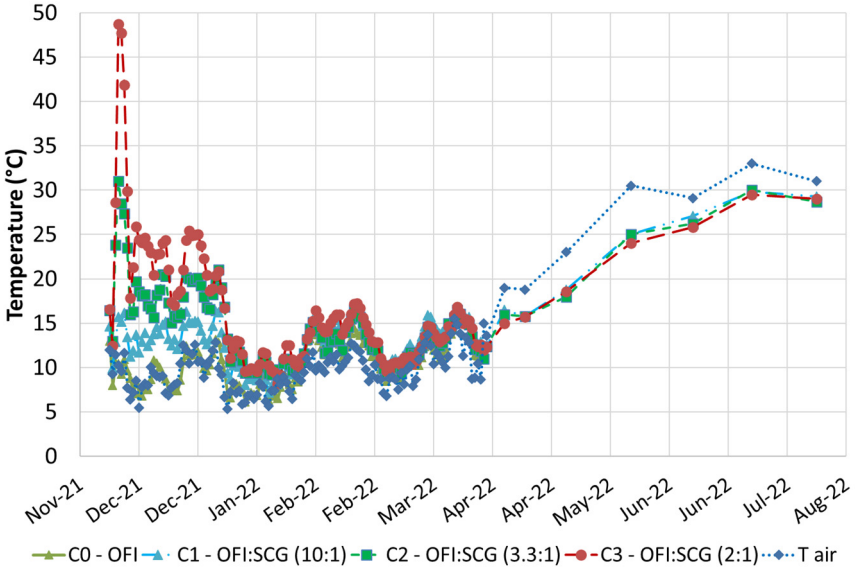

Figure 1. Temperature trend monitored during the composting campaigns for different mixtures. C0 (only OFI), C1 (OFI–SCG—10:1), C2 (OFI–SCG—3.3:1), C3 (OFI–SCG—2:1) and air.

The water content and pH trends reported in Figures 2 and 3 are in accordance with results obtained in the composting trial carried out with OFI pruning, as described in [11].

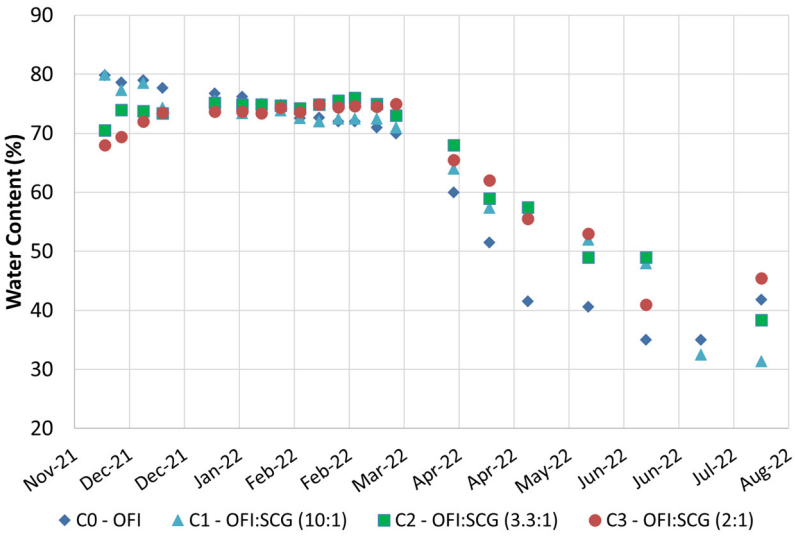

Figure 2. Water content variations during the composting campaigns for different mixtures. C0 (only OFI), C1 (OFI–SCG—10:1), C2 (OFI–SCG—3.3:1), C3 (OFI–SCG—2:1).

The monitored water content appeared to be higher than the known ranges of the composting process (generally between 40 and 60%) with values consistently above 70% for approximately half of the duration of the experiment. pH values showed alkaline conditions in the first half of the trial, with some exceptions for the test with only *Opuntia*.

The carbon to nitrogen ratio is considered a key parameter to evaluate the performances of the composting process. The initial C/N ratio recommended by the literature for all feedstocks is between 25:1 and 40:1. Additionally, keeping a C/N ratio above 15:1 will help ensure nitrogen is not lost and ammonia is not released into the atmosphere [30,31]. As composting proceeds, the C/N ratio gradually decreases, reaching values below 10:1 for the end-product. The C/N ratio for the initial mixtures was above 20, with a slight decrease as the amount of SCGs added increased (Figure 4). This was due to the higher nitrogen content of SCG waste compared to OFI alone. Except for the compost obtained

with only OFI, mixtures C1, C2 and C3 all showed C/N below 10, highlighting the complete biological stability of the compost.

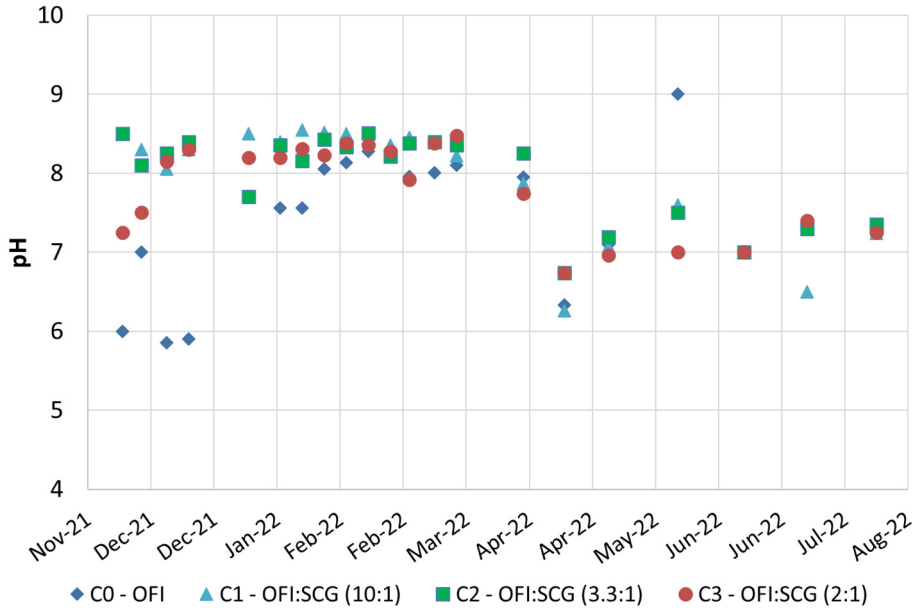

Figure 3. pH variations during the composting campaigns for different mixtures. C0 (only OFI), C1 (OFI–SCG—10:1), C2 (OFI–SCG—3.3:1), C3 (OFI–SCG—2:1).

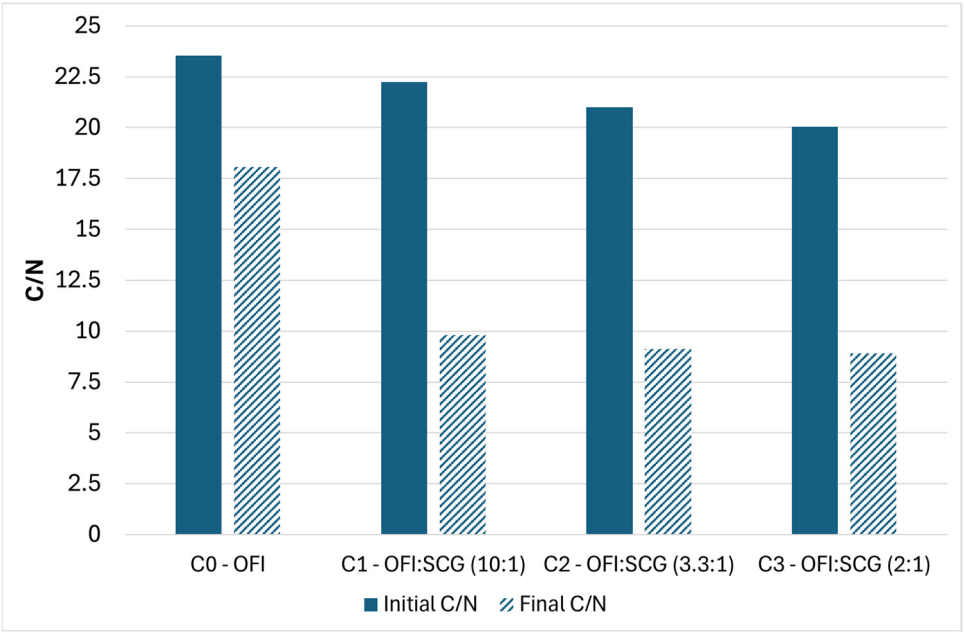

Figure 4. Initial and final C/N ratio for each mixture used for composting campaigns. C0 (only OFI), C1 (OFI–SCG—10:1), C2 (OFI–SCG—3.3:1), C3 (OFI–SCG—2:1).

Metal and major element quantities for raw material OFI, SCGs and composts are reported in Table 3. The values of the concentrations of metals to be analyzed according to Italian legislation, i.e., the heavy metals Cd, Cr (VI), Hg, Ni, Pb, Cu and Zn were under, by at least one or two orders of magnitude, the threshold values admitted in the Italian law on fertilizers [32]. Among the micronutrients analyzed which are essential for plant growth, potassium from raw *Opuntia* showed a concentration higher than 4%, which was also found in the produced composts, with percentages that decreased with respect to the quantity of SCGs added.

Table 3. Chemical composition of raw substrates (OFI and SCGs) before the composting campaigns and final values of compost obtained from different mixtures.

Parameter	*Concentration Limit* Allowed by D. LGS 75/2010	Units of Measure	OFI		SCGs		C0		C1		C2		C3	
			Value	$\pm u_e$	Value	$\pm u_e$	Value	$\pm u_e$	Value	$\pm u_e$	Value	$\pm u_e$	Value	$\pm u_e$
Cd	1.5	mg kg^{-1}	0.042	0.002	<0.020		0.162	0.004	0.145	0.004	0.118	0.004	0.113	0.005
Cr (VI)	0.5	mg kg^{-1}	0.078	0.005	<0.012		0.104	0.004	0.065	0.005	0.043	0.005	0.045	0.004
Mn		mg kg^{-1}	224	9	20.8	1.4	204	9	149	6	141	4	119	3
Hg	1.5	mg kg^{-1}					0.029	0.001	0.033	0.001	0.038	0.005	0.028	0.002
Ni	100	mg kg^{-1}	18.5	1.0	2.86	0.16	8.72	0.24	11.0	0.5	8.4	0.1	10.5	0.4
Pb	140	mg kg^{-1}	0.541	0.043	<0.100		5.42	0.23	3.90	0.16	3.79	0.23	4.33	0.14
Cu	230	mg kg^{-1}	20.6	0.9	22.4	1.6	21.1	0.6	30.8	0.9	32.5	0.7	34.8	0.6
Zn	500	mg kg^{-1}	48.2	3.3	9.03	0.88	261	18	264	7	207	4	200	12
Ca		%	5.78	0.16	0.138	0.006	4.05	0.11	3.86	0.11	3.19	0.03	2.78	0.02
Fe		%	0.008	0.001	0.0048	0.0002	0.296	0.012	0.182	0.008	0.240	0.016	0.175	0.012
P		%	0.175	0.007	0.185	0.010	0.394	0.016	0.691	0.029	0.701	0.030	0.6182	0.0001
Mg		%	0.671	0.019	0.175	0.007	0.517	0.014	0.584	0.016	0.589	0.003	0.539	0.015
K		%	4.92	0.14	0.873	0.036	2.63	0.07	3.23	0.09	3.09	0.09	2.85	0.08

3.2. Basil Growth

Seeds were germinated and seedlings developed both in commercial soil (Ctrl) and in those mixed with compost (C0, C1, C2, C3) (Figure 5). No negative symptoms or other diseases were observed throughout the trial.

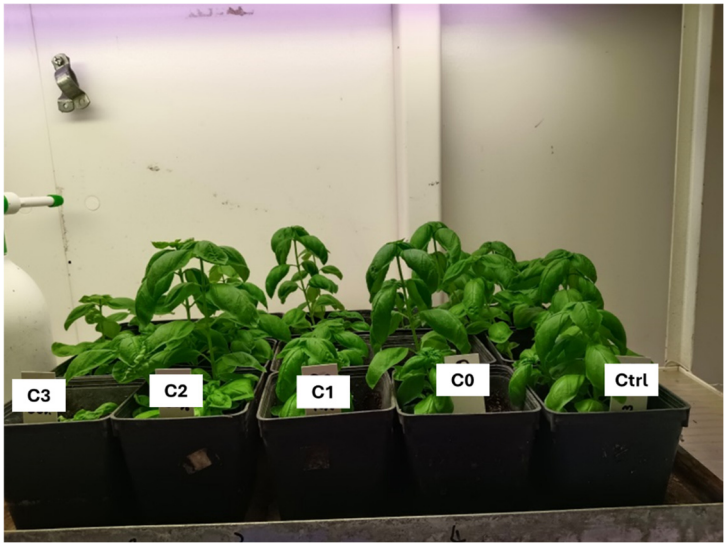

Figure 5. Basil plant growth after 2 months from the sowing. From right to left: only commercial soil (Ctrl); Ctrl and 10% OFI compost (C0); Ctrl and 10% compost (C1); Ctrl and 10% compost (C2) and Ctrl and 10% compost (C3).

Fresh leaf weight, which for many horticultural species is determined mainly by the water content of the harvested product, was higher in plants grown in soil supplemented by 10% of C2 compost after three months of cultivation (Figure 6). The same plants displayed the highest dry weight leaf biomass, showing a higher capacity to allocate biomass for leaf production during the cultivation cycle. These results seem not to have been affected by leaf water content (as a percentage), which was measured to be comparable with plants grown in soil mixed with 10% of the other tested composts. A reduction in leaf moisture percentage was observed at T3 (3 months from sowing) in plants obtained from soil without compost (Ctrl) when compared to those grown in soil mixed with the C0–C3 composts. This was confirmed by the % dry matter, which was inversely proportional to the water content of the sample. The plants obtained by soil supplemented by 10% C2 compost increased proportionally with their % dry matter from T1 to T3 (6.13 $\pm$ 0.5, 6.72 $\pm$ 0.3 and 6.98 $\pm$ 0.3, respectively).

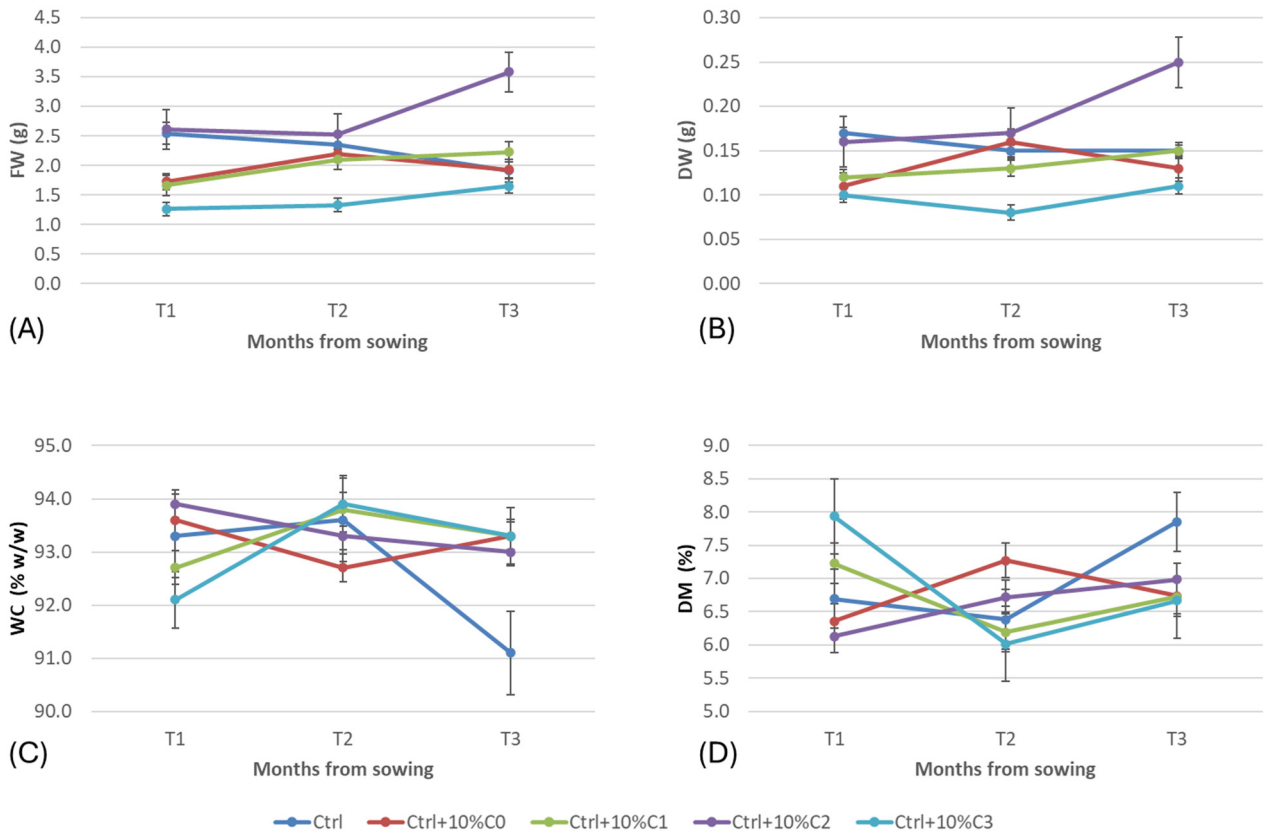

Figure 6. Changes in fresh weight (**A**), dry weight (**B**), water content (**C**) and % dry matter (**D**) in leaf samples collected at the basal part of plants after 1, 2 and 3 months from sowing. The values are the mean of 10 samples ± standard error.

After 4 months of cultivation, no significant differences were found among the root fresh weights of the trials, while a significant difference was found for plant root length when cultivated in soil supplemented by 10% of the C3 compost. In this case, the mean value of root length was about 50% when compared to the control (6.11 ± 1.31 cm versus 11.88 ± 0.90 cm).

Although no significant differences were found among the fresh weights of the plant epigeal parts (Table 4), the shoot biomass showed a significant increase in fresh weight as well as shoot height (+23%) for plants grown in soil supplemented by 10% of C2 when compared with the control. Conversely, plants grown in soil supplemented by 10% of C3 displayed a significantly low number of nodes with expanded leaves.

Table 4. Shoot and root fresh weight, length, height and number of shoot nodes in basil plants after 4 months in control (Ctrl, 0% compost), C0, C1, C2 and C3 soils. The values are the mean of three replications ± standard error.

Plants/Composts	Roots		Shoots		
	Fresh Weight (g)	Length (cm)	Fresh Weight (g)	Height (cm)	Shoot Nodes (n°)
Ctrl	1.58± 0.42	11.88 ± 0.90 a	4.95 ± 0.88 ab	23.72 ± 3.24	8.50 ± 0.37 a
Ctrl + 10% C0	1.28 ± 0.17	14.20 ± 1.47 a	4.26 ± 0.71 ab	19.80 ± 2.65	8.60 ± 0.61 a
Ctrl + 10% C1	2.13 ± 0.72	13.36 ± 1.51 a	4.44 ± 1.08 ab	24.57 ± 5,55	8.43 ± 0.62 a
Ctrl + 10% C2	3.58 ± 1.18	10.16 ± 1.09 a	7.95 ± 1.57 a	30.55 ± 5.68	7.67 ± 0.77 a
Ctrl + 10% C3	0.98 ± 0.37	6.11 ± 1.31 b	2.49 ± 0.91 b	13.35 ± 4.36	5.09 ± 0.88 b
F-value	2.32	0.25	3.42	2.11	4.85
Statistical Significance	0.073	0.001	0.017	0.098	0.003

Different letters represent significant values in accordance with Tukey's Test.

3.3. Total Polyphenol and Flavonoid Content and Antioxidant Activity

The total polyphenol and flavonoid contents in basil plants grown for 4 months in control (Ctrl, 0% compost), Ctrl + 10% C0 or Ctrl with different ratios of OFI/SCG compost (C1, C2 or C3) are shown in (Figure 7A,B). A significant difference was found between polyphenol contents in plants obtained from the control (2.8 ± 0.03 mg GAE g^{-1} DW) and those from soil supplemented by compost (Figure 7A), while no significant difference was found among the polyphenol contents of plants cultivated on soil supplemented by compost. A similar trend was observed for flavonoid content (Figure 7 B); the control plants showed the highest value (22.2 ± 3.3 mgQE g^{-1} DW), which was significantly different from the flavonoid content of plants grown in soil mixed to 10% of C0 or 10% C3 (13.0 ± 2.14 and 10.1 ± 0.9 mgQE g^{-1} DW, respectively).

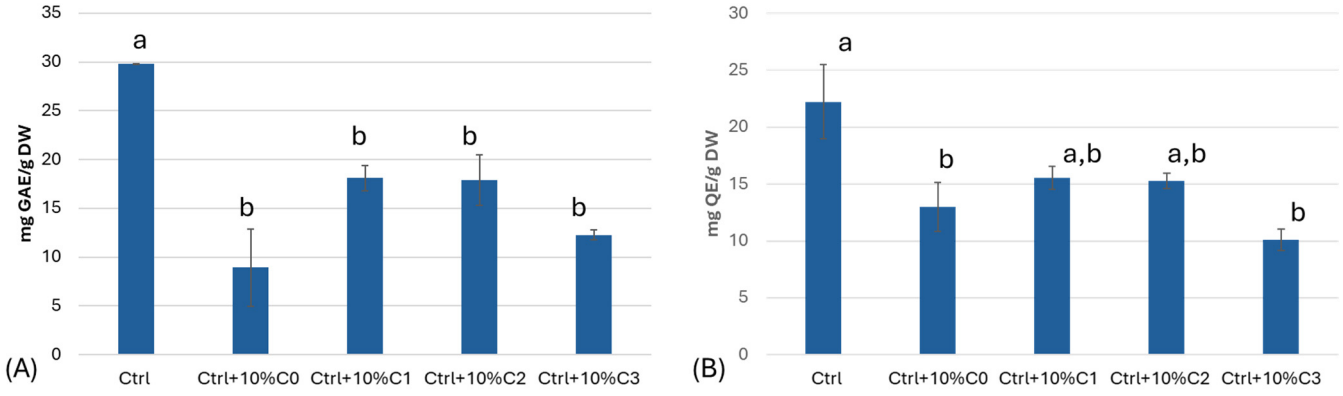

Figure 7. Polyphenol (**A**) and flavonoid (**B**) contents in basil plants grown for 4 months on Ctrl, Ctrl + 10%C0, Ctrl + 10%C1, Ctrl + 10%C2 and Ctrl + 10%C3. The values are the mean of three replications ± standard error. Different letters mean significant values following Tukey's Test.

The antioxidant activity determined by the DPPH test (Figure 8A) was coherent, with the previous colorimetric analysis showing the highest activity in plants from the control (220.8 ± 24.1 µmolTE g^{-1} DW) when compared with those obtained from soil supplemented with compost (Ctrl + 10% C0 = 108.6 ± 15.9, Ctrl + 10% C1 = 172.7 ± 10.7, Ctrl + 10% C2 = 174.9 ± 9.8, Ctrl + 10% C3 = 140.3 ± 12.8 µmolTE g^{-1} (DW)). In the case of iron-reducing activity (Figure 8B), the basil plants grown on Ctrl +10% C2 exhibited similarly high antioxidant activity when compared to those from the control (0.50 ± 12.2 vs. 0.45 ± 15.5 mM$FeSO_4$ eq g^{-1} DW); conversely, for all other cases, reduced activity was observed.

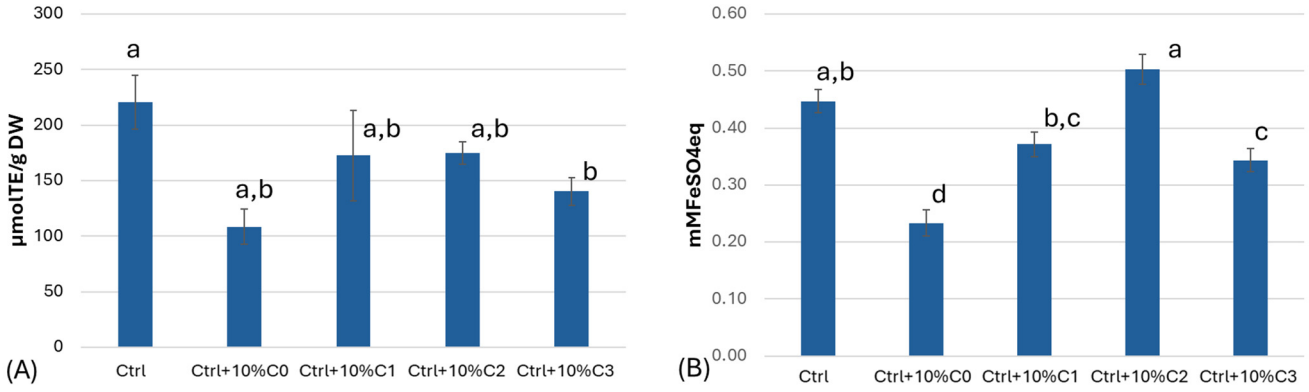

Figure 8. Antioxidant activity in basil plants grown for 4 months on Ctrl, Ctrl + 10%C0, Ctrl + 10%C1, Ctrl + 10%C2 and Ctrl + 10%C3 based on DPPH (**A**) and FRAP (**B**) assays. The values are the mean of three replications ± standard error. Different letters mean significant values following Tukey's Test.

4. Discussion

The results from the composting campaigns confirmed those obtained in [11] and highlighted once again the unique characteristics of *Opuntia* when used as a substrate for composting. Very high water contents for all mixtures were monitored in the first 6 months of experiments; these were considered not suitable for the composting process. Generally, values above 65% lead to anaerobic conditions with the consequent production of toxic gases (e.g., methane and hydrogen sulfide) and odorous emissions. However, in the composting campaigns described herein, none of these emissions were revealed, although they were periodically monitored, as reported in the Supplementary Materials (Figures S1 and S2).

It was speculated, as explained in our previous work [11], that the physical and chemical properties of cladodes increased the metabolic activity of the microbiota involved in the composting process, therefore limiting gas emissions. A hypothesis to understand this behavior may be that the temperature peak remained below 50 °C and the content of proteins in the cladodes was low; both conditions could lead to less ammonia formation and volatilization.

Even the pH trending constantly above neutrality (pH 7) for almost the entire duration of the test and for all three mixtures C1, C2 and C3, albeit with some exceptions, represented an interesting result. Similarly, C0 (OFI only) pH values were slightly below neutrality in the first month, but subsequently this trial also turned to basic conditions. This is remarkable, because the first phase of the composting process is usually characterized by pH values below neutrality due to fatty acid and CO_2 production. OFI's pH-buffer capacity may explain these unusual pH values and it is hypothesized that this property could be used with feedstocks that show a tendency towards acidic pH when composted and could prove to be a decisive buffering characteristic to avoid inhibitory effects [33]. During the curing phase in piles, all three mixtures showed stable pH values around neutrality, as expected.

As a matter of fact, composting microbiota operates best under neutral to acidic conditions. During the initial stages of decomposition, organic acids are mainly formed. The acidic conditions are favorable for microorganisms' growth and for the breakdown of lignin and cellulose. As the composting process proceeds, the organic acids are neutralized, contributing to the increase in pH (6–8) in the mature end-product. The ratio of carbon to nitrogen in the initial material significantly affects pH changes. A high C/N ratio (as in bagasse) can lead to initial acidity as microbes prioritize carbon decomposition.

The interesting features that OFI pruning granted during these composting campaigns (pH-buffer capacity and adsorption capacity) suggest the possibility to successfully use this substrate within in-vessel composting processes with other feedstocks (e.g., food waste). The compost obtained during this study was characterized in the end by a C/N ratio below 10:1. This shift in the C/N ratio occurred because each time that organic compounds were consumed by microorganisms, two-thirds of the carbon was given off as carbon dioxide. The remaining third was incorporated along with nitrogen into microbial cells, then later released for further use once those cells died [31].

Regarding the concentrations of major elements and metals present in composts, the element that provided agronomic value the to compost was manganese (Mn), which is an important micronutrient for plant growth and development and sustains metabolic roles within different plant cell compartments [5,11]. The totality of this element was provided by the cactus pear; in fact, as the quantity of added coffee increased its concentration decreased. A similar feature was visible for potassium (K), with a value close to 5% for raw OFI.

The behavior of the metals zinc (Zn) and iron (Fe) needs to be highlighted, since their concentration increasesd, compared to the initial values present in the OFI and SCGs, by one and two orders of magnitude, respectively. This effect was presumably due to the alkaline conditions created by the buffer capacity of OFI during the composting process [34,35]. The analysis of mineral content determined in basil plants grown on Ctrl and C2 confirmed these results, as reported in the Supplementary Materials (Figure S3).

Other authors reported that SCGs co-composted with cat manure, when applied to soil at 5% (*w*/*w* on a wet basis), improved the height of spinach and did not increase the contents of Zn, Cu, Pb and Cd [36]. Our research on basil plants showed that the applications of *Opuntia* and SCG compost could improve the intake of these kinds of microelements (see Supplementary Materials Figures S4 and S5).

Different authors showed the beneficial effects of organic and bio-fertilizers from agro-industrial residues, which improved soil physical and chemical properties and increased the yield and growth of horticultural crops, including basil [11,37]. Sweet basil (*Ocimum basilicum* L.) is an aromatic species cultivated for fresh market and industrial processing which has become a high-income niche crop for its traditional use and fast production cycle [38,39].

Bacchetta et al. [11] showed that basil plants germinated and grew up without significant morphological differences nor negative symptoms when cultivated in soil supplemented by OFI powder. Furthermore, they demonstrated the effect of the high swelling potential of OFI biomass, due to the chemical structure of the mucilage, which favored water retention in the soil. Furthermore, the utility of utilizing spent coffee grounds as an organic amendment was underlined by a 10-times increase in the soil respiration rate that resulted in an increase in soil organic carbon and total nitrogen contents [40]. As reported by Hu et al. [41], total N in the soil significantly increased with SCG supplementation; however, we noticed a moderate variation in the C/N ratio by increasing SGC content.

Therefore, it is speculated that by combining OFI and SCGs as feedstocks, the compost could exhibit a synergic action to improve soil properties through improvements in nutrient availability, biology fertility and water-holding capacities. Our study indicated that the soil supplemented by 10% compost of C2 improved the quality of basil growth, thus sustaining the above-mentioned hypothesis.

We observed that the basil plants cultivated in these conditions displayed a higher fresh and dry weight leaf biomass, which showed its highest value at plant maturity. During the growing phase, these plants were more efficient in allocating dry matter for yield (leaves) and this higher productivity was evident along the productive cycle. The % water content in the leaves of plants cultivated in the soil supplemented by the different composts was higher than the control when evaluated at plant maturity. As previously reported by Zhou et al. [42], the water content of the fully expanded leaf is an indicator of the soil water status; therefore, the presence of 10% compost contributed to the holding capacity of soil. However, we noted that the compost OFI–SCG (2:1) negatively affected the parameters evaluated in leaf samples, because the fresh and dry weight were the lowest. This effect was more evident when evaluating the plants at the end of the productive cycle. Seedlings from soil supplemented by 10% of C3 were the smallest with the lowest number of nodes and with a significant reduction in the root system. In a different study, the utilization of spent coffee grounds as an amendment limited the growth of lettuces, although it also induced a relative increase in the dry weight (greater degree of mineralization) with possible nutritional benefits of the final product [43]. Moreover, previous studies on the effect of SCGs directly incorporated on agricultural soils confirmed their beneficial action in terms of improving soil's physical, chemical and biological properties [44,45]. However, the composition of SCGs includes high amounts of lipids, with 13–18% [46] responsible for

the hydrophobic nature of this bioresidue. Although the transformation and stabilization of SCGs through composting ameliorate their properties, hydrophobicity and phytotoxicity could represent a limit to crop growth when SCGs are used in inadequate doses [47]. As reported by Hu et al. in their review [41], when SCGs were applied on lettuce (*Lactuca sativa* L.) at low concentrations (e.g., <5% by weight in pot studies and <10 kg m^{-2} in field studies), plant growth was enhanced, whereas it was inhibited by higher concentrations. In general, inhibited plant growth with a high SCG concentration has been primarily attributed to the presence of phytotoxic substances (e.g., caffeine and polyphenols) or SCG-induced nitrogen immobilization, although composting or co-composting can reduce the phytotoxic effects of raw SCGs, as reported by different authors [23,48,49]. As discussed before, improved soil health, particularly improved nutrient availability and soil water retention, is associated with enhanced plant yield and/or quality. These latter considerations can help us to explain our findings on basil chemical composition. Polyphenols, which are commonly detected in plants as secondary metabolites, are generally recognized as molecules involved in stress protection in plants [50]. Therefore, the highest polyphenol content in the leaves of plants from the control (0% of compost) was probably correlated with their lowest soil water retention. The presence of compost made with only OFI or OFI and SCG composts mitigated the water stress in the soil, improving basil growing conditions. Although there were no significant differences between polyphenol and flavonoid content in plants grown on soils mixed with composts, those grown on *Opuntia* compost only showed the lowest content of these biomolecules, while the presence of spent coffee grounds in different percentages induced an increase in polyphenols, although other authors have reported contrasting results on SCG applications [41].

Contradictory results about the relationship between antioxidant production and the addition of a variety of composts were reported in the literature. For example, Verrillo et al. [51] demonstrated an increase in eugenol, eucalyptol and geranyl acetate in basil plants when cultivated on humic substances extracted from green compost made with artichoke biomasses. The results from De la Portilla et al. [52] emphasized that basil's antioxidant capacity decreased in the presence of biosolids. In another study, bio-organic fertilizers enhanced the antioxidant activity due to the high biosynthesis of phenolics, flavonoids and essential oils from basil plant extracts [53]. The same contradictory results were described by Solís-Oba [54] in lettuce when cultivated on agro-waste composts. Furthermore, other agronomic factors can be involved in the synthesis of antioxidants in crops, for example, as reported by Coria-Cayupan et al. [55], nitrogen deficiency induced the accumulation of ascorbic acid and flavonoids in *Arabidopsis* and tomato, while nitrogen availability seemed to reduce total polyphenols and the antioxidant activity. Therefore, regardless of the better water retention, the increase in nitrogen in soil due to the addition OFI and SCG composts could explain the lower antioxidant activity, expressed as the neutralizing capacity of free radicals. On the other hand, the antioxidant activity expressed as the reducing power against a metal ion such as Fe^{+++} was found to be higher in plants cultivated on soil with 10% C2. This seemed to indicate the presence of biomolecules with different mechanisms of antioxidant action (radical scavenge or metal reduction). As suggested by Romano et al. [56], the antioxidant capacities of extracts need to be measured by more than one type of assay to consider their various modes of action. It is known from the literature that *Ocimum basilicum* L. produces several phenolic acids and flavonoids, phenylpropanoids, terpenes (i.e., linalool, bergamotene), tannins and molecules such as rosmarinic, citric, caffeic and ferulic acids [56,57], including various alcohols and aldehydes contained in the essential oils of the leaves [58–60]. It was therefore possible, given the heterogeneity of the various components with antioxidant activity, that the different composts acted on the different

metabolic pathways to different extents by inhibiting the production of compounds which differ from each other in their strength and mechanism of antioxidant activity.

5. Conclusions

This study demonstrated the potential of using *Opuntia ficus-indica* (OFI) cladodes and spent coffee grounds (SCGs) to improve the conditions for the better development of a plant like basil. Composting campaigns revealed that mixtures of OFI and SCGs, particularly at a ratio of 3.3:1, significantly improved soil properties and supported healthy basil plant growth. These composts exhibited beneficial characteristics such as high water retention, nutrient availability and pH-buffering capacity, which are crucial for enhancing soil fertility and crop productivity.

These findings suggested that integrating OFI and SCG compost into agricultural practices can be a sustainable and effective approach to byproduct valorization, contributing to the circular economy and preventing waste production. Further research is needed to explore the long-term effects of these composts on different crops and soil types, as well as their potential applications in larger scale agricultural systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11060640/s1, Table S1. Main characteristics of cured compost used as inoculum. Figure S1. Process monitoring profile for O_2 during the composting campaigns. Figure S2. Process monitoring profile for CO_2 during the composting campaigns. Figure S3. Magnesium, Calcium, Potassium, Phosphor of basil plants after 4 months on control (Ctrl, 0% compost) and C2 The values are the mean of 3 replications ± standard error. Figure S4. Manganese, Iron, Zinc, Copper of basil plants after 4 months on control (Ctrl, 0% compost) and C2 The values are the mean of 3 replications ± standard error. Figure S5. Chromium, Cobalt, Nickel, Arsenic, Cadmium, Lead of basil plants after 4 months on control (Ctrl, 0% compost) and C2 The values are the mean of 3 replications. ± standard error.

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Abbreviations

The following abbreviations are used in this manuscript:

Abs	Absorbance
C	Carbon
C0	Mixture composted with only OFI
C1	Mixture composted with OFI–SCG 10:1
C2	Mixture composted with OFI–SCG 3.3:1
C3	Mixture composted with OFI–SCG 2:1
Ctrl	Commercial soil
DW	Dry weight
DM	Dry matter
FRAP	Ferric Reducing Antioxidant Power
FW	Fresh weight

GAE	Gallic acid equivalents
OFI	*Opuntia ficus-indica*
SCG	Spent coffee ground
T1-T3	Months from sowing
WC	Water content

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