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Composting Dairy Manure with Biochar: Compost Characteristics, Aminopyralid Residual Concentrations, and Phytotoxicity Effects

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Abstract: Aminopyralid (2-pyridine carboxylic acid, 4-amino-3, 6-dichloro-2-pyridine carboxylic acid) is an auxin herbicide that has been used widely to control broadleaf weeds in pasture and hay fields. With no post-application withdrawal time, aminopyralid absorbed into forage material can contaminate compost feed stocks such as hay, grass bedding material, and manure. Composts derived from such feed stocks raises concerns about after-effect injuries to sensitive crops by residual aminopyralids. Biochar (BC) additive may affect the composting process and immobilizes organic pollutants. This study examined the effect of composting dairy manure/sawdust 1:1 mixture containing 10 ppb (wet) of aminopyralid with 0%, 2%, 4%, and 10% (*w*/*w*) BC levels on chemical and biological characteristics of compost, residual aminopyralid concentration, and intensity of plant injury to tomato (*Lycopersicon esculentum* L.) plants after composting in 140 L plastic rotary drum reactors for two 6-month cycles. Biochar addition decreased organic matter degradation and intensified reduction in residual aminopyralid levels in a dose-dependent manner. Composting with BC concentrated more N, P, and K, caused mild plant injuries, and increased the above ground biomass compared to the no BC incorporation. Addition of BC for composting aminopyralid-contaminated dairy manure can increase the phyto safety level of compost while enhancing the key fertilizer values.

Keywords: composting; dairy manure; biochar; herbicide; aminopyralid

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1. Introduction

Herbicidal injury to crops following the application of livestock manure/compost has become a significant concern for producers. Pyridine carboxylic acid herbicides that are widely used in pasture and rangelands have the potential to carryover the effects of herbicides on crops following the application of compost from contaminated feed stocks [1]. Pyridine carboxylic acid herbicide injury to broadleaf crop plants following the application of hay or manure compost has been reported in the United States (US) [2] and across the world [3]. Residual concentrations of pyridine carboxylic acid herbicide even as low as <10 ppb in compost can affect different sensitive broadleaf crop plants [4,5]. Aminopyralid (2-pyridine carboxylic acid, 4-amino-3, 6-dichloro-2-pyridine carboxylic acid) herbicide works as a plant growth regulator and causes auxin-like injuries on plants.

Aminopyralid herbicides have been registered for use on rangelands, pastures, natural areas, noncroplands, rights-of-ways, and riparian areas for controlling annual and perineal broadleaf weeds [6]. Aminopyralid has a favorable human health toxicity profile to be categorized as a low-risk herbicide [7]. The lower application rate and high persistence have made aminopyralid herbicides a favorite choice for broadleaf weed control among livestock producers [5,8]. Further, the low toxicity of aminopyralid herbicide to mammalians make aminopyralid-treated pastures and hay fields quite safe to consume immediately by livestock with no post-application withdrawal time requirement for forage utilization [9].

The residues of aminopyralid herbicides on pasture and hay fields can remain in the environment or in the tissues of contact plant materials for a long period. Research has shown that the half-life of aminopyralid in soil is 34.5 days [10]. Aminopyralid residues have been shown to be stable for 16 months in dry matrices, such as hay and straw [11]. Once forage products from treated fields are ingested, pyridine carboxylic acid (aminopyralid) compounds are quickly eliminated through urine and feces [5] with little absorption and biotransformation [12] and can pass to the manure (a combination of urine and feces) composting process. Given the auxinic mode of action, any aminopyralid residues left in manure compost can injure broadleaf crop plants grown in association with compost material [13]. Cattle manure contaminated with aminopyralid injuring garden plants was reported [14]. After being absorbed by the roots, aminopyralid is translocated throughout the plant, inducing an auxin-type response in susceptible plant species, causing epinastic bending and twisting of leaves and stems, deformation of leaves, cupped or elongated leaves, and stunting of roots that results in growth inhibition [8,15]. At low concentrations, auxin can stimulate growth through cell division and elongation [16].

Composting is a process of bio-decomposition and stabilization of organic matter through the action of diverse microorganisms under aerobic conditions [17]. Composting dairy manure has become a popular alternative manure management method that results in manure stabilization, mass and moisture reduction, and reduction in pathogen levels [18]. Composting manure itself can be very inefficient due to high moisture content, low porosity, and low C: N ratio [19]. Therefore, substrate conditioning with a bulking agent that improves C:N ratio, regulates moisture content, and enhances porosity, structure, and oxygen diffusion [20] is required for optimal composting of animal manure. Bulking agents are commonly fibrous with carbonaceous material with low moisture content [21]. Of the cellulosic agricultural and forestry by-products, sawdust as a bulking agent has been found to be significant in reducing moisture content [22].

Biochar (BC), a solid material produced by thermo-chemical conversion of biomass under oxygen-limited conditions [23], has high porosity that can enhance aeration and stimulates microbial growth during composting [24]. Biochar has also become one of the preferred carbonaceous bulking materials for co-composting with manure [25]. The addition of biochar for composting offers an extra benefit as a smart adsorbent [26] for organic pollutants [27,28] including herbicides [29]. Biochar produced at relatively high pyrolysis temperatures is generally effective in the sorption of organic contaminants [23], which is attributed to the physicochemical properties, such as large surface area, porosity, and surface chemistry [30].

Composting itself can degrade most herbicides and insecticides [31,32]. It has been reported that clopyralid concentrations declined from 32 ppm to <1.4 ppm after 365 days of composting grass clippings, but the levels were still 10 to 100 times above the no-observable-effects level [33]. The general pattern of herbicidal degradation while composting has been found to be similar to the degradation observed in the soil environment [32], where soil microbes are quite effective at metabolizing herbicides into permanently inactive forms where they can be a major factor in determining the overall fate of herbicide compounds [34]. Enzymatic transformation, which is mainly the result of biotic processes mediated by plants and microorganisms, is by far the major route of detoxification [35].

The addition of biochar for composting offers an extra benefit as a smart adsorbent for organic pollutants including herbicides. Biochar produced at relatively high pyrolysis temperatures is generally effective in the sorption of organic contaminants, which is attributed to its physicochemical properties, such as large surface area, porosity, and surface chemistry.

The bioassay process uses sensitive plants to detect herbicide contamination of manure/compost. The plant bioassay method for aminopyralid uses sensitive broadleaf crop plants, such as tomato (*Lycopersicon esculentum* L.) and peas (*Pisum Sativum* L.), to determine visual plant injury symptoms and to indicate the presence of herbicide residues in compost [36]. Although bioassay tests are less specific than chemical tests, they are

much less expensive and can be useful in detecting phytotoxic metabolites that are not detected in chemical tests. However, improper composting can also result in compost quality characteristics that cause phytotoxicity symptoms similar to the pyridine carboxylic herbicides [37]. Salinity in growing media has been shown to exacerbate harmful effects for tomato- and cucumber-like crop plants [38], which are frequently used as test plants for herbicide bioassays [39]. High salinity affects the water relations of the plant, which may cause yield reduction, leaf burning, and leaf deformation. Salinity levels between 1.99 and 3.5 dSm^{-1} are commonly accepted as favorable for seedling growth [36]. In addition, immature compost may contain high salt concentrations, volatile organic acids, fatty acids, phenolic acids, and ammonia in sufficient quantities to cause stunting and other phytotoxic symptoms [37]. As such, utmost care is needed to accurately distinguish plant damage from residual herbicide and from other factors of compost.

Not many compost studies have been conducted in small-scale compost reactors. However, pilot-scale composting studies conducted in small reactors enable easier tracking of the composting process than in full-scale [40]. Reactors of 10–300 L size frequently involve a self-heating phase that depends solely on microbial heat production and ensures a well-conducted composting process. Under such conditions, the simulation of the thermodynamic regime (thermophilic, cooling, and maturation phases) has enabled the reproduction of many parameters of full-scale composting systems, including biological activity and metabolic capacities [41,42].

There has been limited information evaluating the composting of dairy manure contaminated with aminopyralid with biochar in small-scale reactors for managing residual aminopyralid concentrations, associated phytotoxicity effects, and the chemical and biological characteristics of compost. A better understanding of the effect of biochar addition on composting dairy manure contaminated with aminopyralid is significant for managing residual herbicidal effects on sensitive crop plants and designing manure composting systems that ensure the production of biologically safer compost products with high fertility characteristics. Such information would provide a solid scientific base for promoting the safe use of manure compost originated from aminopyralid-contaminated feed stocks widely in agricultural production.

The primary objective of this study was to investigate the effect of biochar (BC) addition at 0%, 2%, 4%, and 10% rates for composting dairy manure + sow dust 1:1 mixture containing 10 ppb (*w*/*w*) aminopyralid on the chemical and biological characteristics and residual aminopyralid concentrations of compost and the phytotoxicity effects of dairy manure compost on tomato plants after 6-month composting periods. The hypotheses of this study were based on the fact that addition of biochar to dairy manure composting starter mixtures alters (i) the basic chemical and biological properties of dairy manure compost and (ii) the aminopyralid residual concentrations and intensity of plant injury to tomato plants.

2. Materials and Methods

This study was conducted at the Agriculture Research and Education Complex (AREC), Department of Agriculture and Food Science, Western Kentucky University, Bowling Green, KY, USA. as two independent runs, each lasting for a 6-month period from Aug. 2022 to March 2023 (cycle 1) and from March 2023 to Sept. 2023 (cycle 2) under natural environmental conditions.

2.1. Compost Starter Mixtures, Reactors, and Composting Procedure

The compost starter mixtures were prepared in a similar manner for the two composting cycles by mixing 22.5 kg of fresh dairy manure (86–88% moisture) collected freshly from the manure pit of the dairy barn of AREC with an equal amount (22.5 kg) of sawdust (8.2–8.8% moisture, 1.2 g N kg^{-1}, 385.2 g C kg^{-1} and 355:1 C/N ratio). The dairy herd from which the manure originated consisted of 53 Holstein Friesian milking cows managed in a

free stall barn setting. The manure in the pit contained direct scrapes from the alleyways of the dairy barn with no bedding material.

It is considered that a C/N ratio of 25:1 to 30:1 and a moisture content of 60–65% is optimal for the composting process [43,44]. Several preliminary pilot trials conducted to determine the manure/sawdust combination that adjusted the moisture content to 60–65% level revealed that mixing of 1:1 dairy manure and sawdust achieved the desired moisture level. Pretesting of 1:1 dairy manure and sawdust mixture for C/N suggested an addition of 1.8 g mineral N kg^{-1} compost starter mixture to adjust the final C:N ratio to the desired level of 30:1 and to avoid the limitation of organic matter decomposition by low mineral N availability. Thereafter, compost starter mixtures (45 kg) were mixed with 0%, 2%, 4%, and 10% (*w*/*w*) biochar (BC) rates to formulate 0% BC, 2% BC, 4% BC, and 10% BC biochar treatments. Biochar used in this experiment was produced from pyrolysis of softwood at 500–600 °C (Biochar Now, CO, USA).

To examine the relationship between herbicide concentration and the response of transplanted 10-day-old tomato plants (*Lycopersicon esculentum* L.), a series of preliminary bioassays were conducted using a potting mix containing 1, 10, 20, 30, 40, 50, and 100 ppb levels of aminopyralid. Results from this study indicated 10 ppb as the minimum level causing injuries to tomato test plants. Accordingly, we selected 10 ppb aminopyralid level to contaminate dairy manure compost starter mixtures. Aminopyralid of 99% purity (Sigma–Aldrich Inc. St. Louis, MO, USA) was dissolved in deionized water and, then, mixed thoroughly with dairy manure to give 10 μg kg^{-1} concentration in the dairy manure + sow dust + BC mixtures.

The compost starter mixtures of four BC treatment mixtures were filled into sixteen 140 L plastic rotary drum reactors (four replicates per treatment) and composted under natural environmental conditions. To aerate and homogenize the material and to promote composting, the reactors were rotated twice in the first week and, thereafter, once a week. The rotation of reactors was stopped when the temperature inside the compost decreased slowly to reach ambient temperature. To maintain the microbial activity, the moisture content of the compost materials was monitored biweekly, and optimum 50–60% moisture levels [45] were ensured by adding water. The temperature within the compost material was recorded weekly for the first four months using a hand-held 0.9 m compost temperature probe (Reotemp, San Diago, CA, USA) at the center of the compost material within the reactors. Each composting cycle (1 and 2) lasted for a 6-month period before sampling the compost for various analyses.

2.2. Compost Sampling, Chemical and Biological Analysis

The compost starter mixtures within the reactors were sampled twice, one at the beginning of the experiment on day zero and the other at the end of each 6-month period. Before drawing samples, the material inside was homogenized by rotating the reactors and, thereafter, by protected (using separate hand gloves) manual mixing. The sample collected for chemical analysis of aminopyralid concentration and chemical and biological characteristics of composts weighed 500 g. Another compost sample weighing 3 kg was collected for use in bioassays. The compost samples collected for chemical analysis and bioassay tests were stored at 4 °C under refrigeration until the respective analysis/test was performed, while the samples collected for biological analysis were stored at −80 °C.

The pH, electrical conductivity (EC), ash, total nitrogen (TN), total carbon (TC), NO_3-N, NH_4-N, organic matter (OM), potassium (K), and phosphorus (P) were determined according to the standard protocols specified by the US Composting Council (TMECC, 2002). Sub-samples were ground to the particle size specified by the analytical method for each chemical property. Moisture content (*w*/*w*) was determined after oven drying (60–80 °C) to a constant weight. The pH and electrical conductivity of compost material was determined according to TMECC method 04.11-A and using a solu-bridge conductivity meter (Beckman Instruments, Cedar Grove, NJ, USA) according to TMECC method 04.10-A. After heating for 4 h in a muffle furnace at 550 °C, ash content in the compost was determined according

to TMECC method 03.02-A. Total nitrogen (N) and total carbon (C) analyses were performed (Dumas combustion method) using Vario Max CN analyzer (Elementar America Inc., Mt. Laurel, NJ, USA) (TMECC methods 04.02-D and 04.01-A). Total nitrate–N and ammoniumN contents were determined by KCl extraction and flow-injection colorimetric analysis on a Lachat Quickchem FIA+ 800 analyzer (USA Hach Co., Loveland, Colorado) according to TMECC method 04.02- B & C. Total organic matter (dry basis) content was determined by loss on ignition method (TMECC method 05.07-A). Total K and P were measured by ICP after microwave digestion of 0.1 g of oven dried sample with 4 mL 69% HNO3 and 1 mL 33% H_2O_2 [46]. The total DM and OM loss during composting was assessed for cycle 2. To determine initial reactor mass and mass loss during composting, composting mixtures in each reactor were weighed at the start (day zero) and at the end of the 6 m period. The assessments were conducted as the difference of total DM or OM contents in the initial and final masses as a percentage of initial mass.

Total community DNA was extracted from 300 mg of the initial mix and final compost samples using the Dneasy PowerSoil Pro Kit in a QIAcube semi-automated instrument (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. Quantitative PCR run was performed using CFX96 instrument (Bio-Rad, Hercules, CA, USA). Bacterial 16S rRNA was amplified using published primer and TaqMan probe sequences (Table 1). The PCR mix of 25 µL total volume consisted of 10 µL of probe master mix (nodUTP; Bio-rad), 1 µL of primer pairs (10 µM concentrations), 1 µL of TaqMan probe (5 µM), 11 µL of PCR clean water, and 1 µL sample DNA. The PCR program was 95 °C for 15 min, 40 cycles at 95 °C for 15 s, 58 °C for 45 s, and 72 °C for 45 s. The 16S rRNA gene used as a PCR standard was *E. coli* 25922 DNA cloned into TOP TA (3908 bp) vector (Invitrogen, Waltham, MA, USA) using 16S-27F/16S-1492R with the resulting insert size of 1465 bp. A 10-fold serial dilution of the 16S rRNA standard ranging from 101 to 108 was used. The primers and probes were obtained from Integrated DNA Technologies, Inc. (Coralville, IA, USA).

Table 1. Sequences, target size, and annealing temperature of primers used in this study.

Primer	Primer Sequence (5′-3′) †	Tm ‡ (°C)	PCR Product (bp) §
Forward	ATGGCTGTCGTCAGCT	58	337
Reverse	ACGGGCGGTGTGTAC		
Probe	CAACGAGCGCAACCC		

† Probe sequences each contained a 5′ FAM fluorophore and 3′ Black Hold Quencher combination for use in probe-based 5′ nuclease assays; Probe concentration of 200 nM; Primer concentration of 400 nM; ‡ Tm. (°C) is the annealing temperature of the PCR reaction; § PCR Product refers to the expected amplification product size in nucleotide base pairs (bp).

2.3. Chemical Analysis for Aminopyralid Residual Concentration

Determination of aminopyralid concentration in compost was performed by an analytical laboratory (Anatek Labs, Moscow, ID, USA) using Shimadzu LC-20AD Liquid Chromatograph (Shimadzu Scientific Instruments, INC., Redwood City, MD, USA) coupled with a Sciex API 4000 Mass Spectrometer (Sciex, Illinois, CA, USA) and a Shimadzu SIL020A HT auto sampler, using Sciex Analyst software (X500 QTOF). Compost samples were prepared by weighing approximately 10 g of sample into a 250 mL plastic bottle. Hundred mL of an extraction solution (70 g NaOH and 710 g NaCl diluted to 15 L with DI water) was added, and samples were tumbled for 1 h. A 2 mL aliquot was taken into a 15 mL centrifuge tube, 2 mL 2N HCl was added, and the tube was vortexed, then heated for 90 min at 80 °C. Samples were then centrifuged prior to cleanup. The samples were purified using Oasis HLB and Oasis MAX cartridges (Waters Corp., MA, USA). For derivatization, extracts were reconstituted with 200 µL derivatizing coupling reagent acetonitrile/pyridine/butanol (22:2:1). Samples were derivatized by adding 10 µL of butyl chloroformate reagent and letting stand 5 min with gentle mixing. Thereafter, 790 µL of the Sample Solvent (water/MeOH—60:40 with 0.05% formic acid and 5 mM ammonium formate) was added prior to analysis on the HPLC. The average recovery level of the

method was 94.8% with a %RSD of 25.7%, and any interferences were negligible. The limit of quantification (LOQ) and detection (LOD) on the equipment were 5 ppb and 1 ppb, respectively.

2.4. Bioassay Tests

The relationship between residual herbicide concentration and plant response was determined by bioassay tests using potted tomatoes (Lycopersicon esculentum, 'cherry red'). The bioassay tests were conducted in a greenhouse with 25/18 °C day/night temperature and without artificial lightening. The compost materials from cycle 1 (6 months old) were mixed with potting soil (1:3) before being used for bioassay tests in 0.75 L pots. For the bioassay test, ten 0.75 L pots containing composts and potting mixture (1:3) from cycle 1 were transplanted to one tomato plant of phase 13–19 BBCH. The pots planted with tomato plants were placed on plastic saucers to collect any excess water drain. The pots were watered 1–3 times per week to ensure water holding capacity but to minimize leaching. Individual tomato plants in pots were grown until phase 21 of BBCH scale (after 2 months) and then, damage to tomato plants was evaluated. The injury level of plants, height of plants measured from the root collar to the top of the plant (cm), and dry weight of the aboveground part (g plant^{-1}) after 2 m were evaluated for the test plants. The visual injury score assessment was confined to the top three leaves from the growing point of each test plant. A point scale (1–4), 1—without injury; 2—cupping; 3—twisting; 4—drying leaf, was established to assess the intensity of plant damage. Mean injury levels per plant were calculated accordingly.

2.5. Experimental Design and Data Analysis

Two-factor factorial completely randomized design (CRD), with BC treatment and composting cycle as the two factors, was used in this experiment. The properties of initial compost starter mixtures were used as covariate when analyzing for the chemical and biological properties of compost. The biological characteristics of compost were assessed by the number of 16S rRNA gene copies. Before statistical analysis, 16S rRNA gene copy numbers were normalized with log-transformation. Effect of BC treatment on measured variables and comparisons of treatment means was performed by ANOVA GLM procedure followed by Tukey's honest significant differences test at 5% level of significance in IBM SPSS 29 (IMB Corp., Armonk, NY, USA). When treatment × composting cycle interaction effects were found to be significant, a separate analysis for treatment effect was performed for each composting cycle; otherwise, averaged values for the two cycles were used for the analysis.

3. Results

3.1. Environmental Conditions

The ambient temperature profiles for the two composting cycles are presented in blue within Figures 1 and 2. There were comparatively higher mean ambient temperatures at the time of compost temperature measurement (between 9.00 am and 10.00 am) during the early period of composting cycle 1 compared to cycle 2. The maximum mean ambient temperature for the two cycles exceeded 25 °C and were 26.1 °C and 29.4 °C, respectively. During cycle 1, minimum mean daily ambient temperature was 0.6 °C, and it was 9.4 °C for cycle 2. The mean daily ambient temperatures reported during cycles 1 and 2 were 17.1 °C and 21.7 °C, respectively.

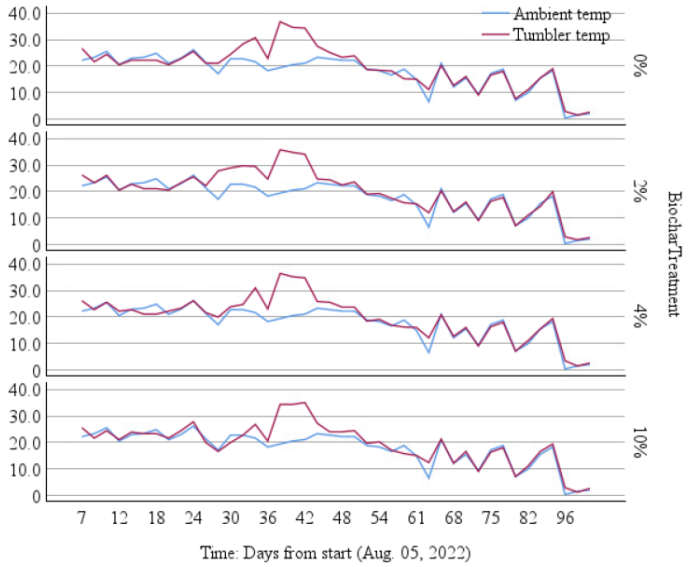

Figure 1. Ambient temperature (°C) and temperature measured within the compost material (°C) between 9.00 and 10.00 am for biochar treatments during composting cycle 1. Values represent the mean of four reactors per treatment per day.

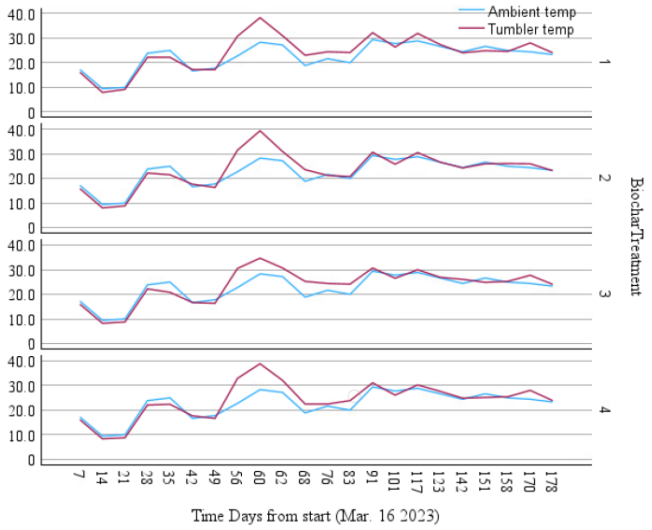

Figure 2. Ambient temperature (°C) and temperature measured within the compost material (°C) between 9.00 and 10.00 am for biochar treatments during composting cycle 2. Values represent the mean of four reactors per treatment per day.

3.2. Composition of Dairy Manure and Compost Starter Mixtures

The mean composition values of dairy manure used for the two composting cycles given in Table 2. The results showed no considerable differences between them. The two sets of dairy manure had neutral or near neutral mean pH but reported relatively higher mean EC than the optimal amount for plant growth and ranged from 6.7 (cycle 2) to 8.1 (cycle 1) ds m^{-1}. The dairy manure exhibited a typical low mean C/N value that ranged from 13.6 to 18.7 and contained a relatively high moisture level (861–879 g kg^{-1}), around 21 g kg^{-1} ash, and 100–116 g kg^{-1} organic matter, on average. The mean NH_4-N content in the dairy manure was low (1 g kg^{-1}) but contained exceptionally high amounts of NO_3-N (42–50 g kg^{-1}) compared to the typical undetectable levels. The mean total N content varied between 3.6 and 4.2 g kg^{-1}, and the greater portion appeared as organic N (2.2–3.1 g kg^{-1}). In addition, dairy manure contained relatively low amounts of P (0.6–0.8 g kg^{-1}) and 3.3 g kg^{-1} K content.

Table 2. Composition of dairy manure used for two composting experiments.

Parameter	Cycle 1	Cycle 2
pH	6.7	7.0
Conductivity (ds m^{-1})	8.1	6.7
C:N ratio	18.7:1	13.6:1
	g kg^{-1}	
Moisture	861.8	879.1
Ash	21.4	20.5
Organic matter	116.8	100.4
Total N	3.63	4.28
NH_4-N	1.4	1.2
NO_3-N	50.0	42.8
Organic N	2.2	3.1
P	0.8	0.6
K	3.3	3.4

The composition of the compost starter mixtures with different BC levels is presented in Table 3. Except the moisture (cycle 1) and ash contents (both cycles), other chemical and biological properties of the compost starter mixtures were uniform among the BC treatments. The moisture content of compost starter mixtures varied between 697 and 739 g kg^{-1}. The compost starter mixtures exhibited slightly basic nature with the mean pH values fluctuating around 8.3–8.9. The C/N ratio of compost starter mixtures from cycle 1 were comparatively lower and ranged between 27.2 and 29.8 as compared to the 36.9–43.3 in cycle 2. The EC of the compost starter mixtures differed considerably between the two cycles with 2.8–3.4 ds m^{-1} in cycle 1 and 5.4–6.9 ds m^{-1} in cycle 2, on average. The highest ash contents were observed in the 10% BC treatment (20.5–33.7 g kg^{-1}), whereas the lowest values of 13.3 and 14.8 g kg^{-1} were detected in the 0% (cycle 1) and 2% (cycle 2) BC treatments. The organic matter (OM) levels ranged between 247.5 (cycle 2, 0% BC) and 287.3 (cycle 2, 10% BC) g kg^{-1}, and organic C (OC) levels ranged between 127.8 and 164.3 g kg^{-1}. In general, the treatments of cycle 1 contained higher total N and the other nitrogen components (NH_4-N and NO_3-N) than in cycle 2. Total N content in cycle 1 varied between 5 and 6 g kg^{-1} compared to the 3.0–4.0 g kg^{-1} in cycle 2. The compost starter mixtures of cycle 1 reported NH_4-N contents > 2 times as much as the contents of the treatments of cycle 2. While NO_3-N (except the control manure mixture) was undetectable in all the compost starter mixtures from cycle 2, 40–48 g kg^{-1} concentrations were detected in cycle 1. The P and K contents did not vary considerably between the two cycles and fluctuated around 0.4 g kg^{-1} and 2.0 g kg^{-1}, respectively.

Table 3. Chemical and biological characteristics of compost starter mixtures of biochar treatments (BC) from the two composting cycles.

	Cycle 1				Cycle 2			
Parameter	**0% BC**	**2% BC**	**4% BC**	**10% BC**	**0% BC**	**2% BC**	**4% BC**	**10% BC**
log16SrRNA	10.0 ± 0.1	10.1 ± 0.1	10.2 ± 0.1	10.1 ± 0.1	9.4 ± 0.1	9.4 ± 0.1	9.5 ± 0.2	9.5 ± 0.1
pH	8.8 ± 0.06	8.9 ± 0.03	8.8 ± 0.05	8.8 ± 0.03	8.3 ± 0.1	8.3 ± 0.1	8.4 ± 0.1	8.5 ± 0.1
C:N	27.5 ± 0.7	27.2 ± 1.9	29.8 ± 1.0	27.6 ± 2.8	36.9 ± 3.3	43.3 ± 3.5	40.7 ± 6.5	39.1 ± 3.7
		ds m^{-1}				ds m^{-1}		
EC	3.2 ± 0.6	3.2 ± 0.5	2.8 ± 0.5	3.4 ± 0.3	5.4 ± 0.4	6.9 ± 0.4	5.6 ± 0.6	6.8 ± 0.4
		g kg^{-1}				g kg^{-1}		
Moisture	731.8 ± 2.5 a	711.5 ± 8.0 ab	697.7 ± 7.4 b	704.9 ± 7.3 ab	739.7 ± 3.3	731.0 ± 15.4	720.5 ± 16.4	699.1 ± 22.6
Ash	13.3 ± 1.8 a	16.0 ± 1.2 ab	19.1 ± 2.4 ab	20.5 ± 0.6 b	19.0 ± 3.6 a	14.8 ± 6.1 a	23.9 ± 1.7 ab	33.7 ± 2.0 b
OM	255.0 ± 4.1	272.5 ± 8.7	283.2 ± 7.2	274.6 ± 7.0	247.5 ± 4.4	256.1 ± 10.4	255.6 ± 17.0	287.3 ± 20.6
OC	147.9 ± 2.4	158.1 ± 5.1	164.3 ± 4.2	159.3 ± 4.0	128.9 ± 7.1	128.0 ± 5.2	127.8 ± 8.5	133.6 ± 10.3
Total N	5.4 ± 0.2	5.9 ± 0.4	5.5 ± 0.2	5.9 ± 0.5	3.6 ± 0.6	3.0 ± 0.1	3.3 ± 0.3	3.4 ± 0.1
NH_4-N	3.3 ± 0.1	3.6 ± 0.2	3.2 ± 0.2	3.5 ± 0.9	1.2 ± 0.6	0.9 ± 0.1	1.3 ± 0.5	1.0 ± 0.1
NO_3-N	45.0 ± 2.9	48.0 ± 2.5	40.0 ± 4.1	45.0 ± 6.5	12.5 ± 12.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Org. N	2.1 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.4 ± 0.4	-	-	-	-
P	0.4 ± 0.02	0.4 ± 0.03	0.5 ± 0.08	0.5 ± 0.07	0.4 ± 0.05	0.5 ± 0.04	0.5 ± 0.1	0.5 ± 0.06
K	1.9 ± 0.06 a	2.1 ± 0.04 ab	2.4 ± 0.2 ab	2.5 ± 0.1 b	2.3 ± 0.1 a	2.4 ± 0.2 ab	2.3 ± 0.2 ab	2.4 ± 0.2 b

EC, Electrical conductivity; OM, organic matter; OC, organic carbon; BC, biochar. Mean ± SE. Numbers with different letters within cycles across columns are significantly different at $p < 0.05$.

3.3. Composting Process

The temperature evolutions at the time of measurement within the composting material during the two composting cycles are presented in Figures 1 and 2. During the two composting cycles, the maximum temperatures recorded by compost material of any treatment ranged between 39.4 °C and 40 °C, and the minimums were reported as 1.1 °C, and 7.9 °C in cycle 1 and cycle 2, respectively. The highest difference between the temperature within compost and ambient temperature by any treatment for cycle 1 and 2 was noted as 20.6 °C and 11.1 °C, respectively.

The total dry matter (DM) mass loss, mass and percentage mass change (per kg DM) of organic matter (OM), organic C (OC), total N, P, and K during composting cycle 2 are presented in Table 4. Although the initial total DM content did not vary significantly among the BC treatments, it was slightly higher in the 10% BC treatment. During the composting process, 0%, 2%, and 4% BC treatments lost comparatively higher amounts of total DM mass (5–6 kg). The lowest amount 3.5 kg of DM loss was noted in the 10% BC treatment. A similar dose–response trend was noted for the loss of OM and OC (per kg DM) among the treatments. The total N, P, and K were concentrating in the compost (N: 4.3–50.9%; P: 7.7–26.5%; K: 5.2–25%) with highest aggregation of P and K occurring in the 0% BC treatment. The aggregation of N increased with the increase in BC concentration in the treatments showing a dose–response relationship.

Table 4. Total mass and percentage mass change in dry matter (DM), organic matter (OM), organic carbon (OC), total nitrogen (TN), P, and K in the compost from initial total content over the course of composting cycle.

Parameter	0% BC	2% BC	4% BC	10% BC
		kg		
Total initial DM	11.4 ± 0.1	11.8 ± 0.6	12.1 ± 0.6	13.4 ± 0.8
		Mass change		
Total DM	6.4 ± 0.4 a	5.0 ± 1.1 a	5.2 ± 1.0 a	3.5 ± 0.8 b
		g kg DM^{-1}		
OM	515.7 ± 32.9 a	434.7 ± 33.6 a	377.8 ± 53.5 ab	188.9 ± 79.9 b
OC	272.3 ± 15.6 a	217.3 ± 16.8 a	188.9 ± 26.8 ab	93.3 ± 41.0 b
TN	0.0 ± 1.7 a	−3.9 ± 1.4 ab	−4.9 ± 0.3 b	−5.6 ± 0.7 b
P	−0.3 ± 0.2	−0.1 ± 0.1	0.4 ± 0.8	0.2 ± 0.2
K	−1.5 ± 3.5	−0.3 ± 0.4	−0.4 ± 0.6	−1.2 ± 0.6
		% Mass change from initial		
DM	56.1 ± 3.3 a	40.3 ± 6.5 ab	40.7 ± 5.8 ab	18.0 ± 10.8 b
OM	56.0 ± 3.5 a	46.1 ± 5.5 ab	44.5 ± 5.8 b	20.1 ± 11.5 b
OC	29.9 ± 0.8 a	23.1 ± 2.7 ab	22.2 ± 2.9 ab	9.9 ± 5.9 b
TN	−4.3 ± 10.1 a	−38.1 ± 14.2 ab	−45.1 ± 5.8 ab	−50.9 ± 9.8 b
P	−26.5 ± 21.1	−10.8 ± 7.9	−7.7 ± 44.2	18.0 ± 21.6
K	−25.0 ± 48.3	−5.2 ± 5.8	−6.4 ± 7.3	−18.9 ± 10.8

Mass change = (initial total (dry) − final total (dry)); % Mass change = (initial total (dry) − final total (dry)) as a percentage of initial total; BC, biochar; Mean ± SE. Numbers with different letters within rows for BC treatments are significantly different at $p < 0.05$.

3.4. The Biological and Chemical Characteristics of Compost

The biological and chemical characteristics of compost for the BC treatments are presented in Table 5. The initial 16S rRNA gene copy numbers among the treatments were similar for the two cycles (Table 2). The final mean log 16S rRNA gene copy numbers, as well, remained similar among the treatments. However, the gene copy number in the treatments with BC was slightly higher (by 0.2 order) than the control treatment with no BC. The final compost materials were slightly basic pH (7 < pH < 8). The C/N ratios of composts exceeded 15:1 but did not exceed 20:1 optimum except the 25.7:1 found in the 10% BC treatment of cycle 1. The compost materials showed low EC values that ranged between 2 and 3 ds m^{-1}. The moisture content of composts exceeded 500 g kg^{-1} level for almost all the samples and did not differ among the BC treatments. Biochar addition increased the

ash and OM contents of composts compared to no BC addition almost in a dose–response manner. However, the ash and OM contents in cycle 1 differed significantly between the 10% level and 4–10% BC levels. For both cycles, total N content did not vary significantly among the treatments and ranged between 7.1 and 9.5 g kg^{-1} in cycle 1 and between 10.1 and 13.9 g kg^{-1} in cycle 2. The NH_4-N/NO_3-N ratio of composts too did not differ among the treatments for the two cycles, but the ratio was slightly higher in the compost from cycle 1 than from cycle 2. Similar concentrations of P and K were detected in composts from all the BC treatments during cycles 1 and 2. The P levels within the two cycles varied from 0.8 to 1.9 g kg^{-1}, but there were slightly higher K levels in cycle 2 (5.5–10.7 g kg^{-1}) than in cycle 1 (3.3–4.6 g kg^{-1}).

Table 5. The mean 16S rRNA gene copies and chemical characteristics of compost of biochar (BC) treatments during composting cycles 1 and 2.

Parameter	n	0% BC	2% BC	4% BC	10% BC
log 16S rRNA gene copies g^{-1}	8	10.1 ± 0.1	10.3 ± 0.1	10.3 ± 0.1	10.3 ± 0.0
Cycle 1					
pH	4	7.8 ± 0.0	7.6 ± 0.2	7.4 ± 0.0	7.8 ± 0.1
C:N ratio	4	19.2 ± 0.8 a	20.8 ± 2.2 a	20.3 ± 1.3 a	25.7 ± 0.8 b
				dsm^{-1}	
EC	4	1.9 ± 0.1	2.8 ± 0.3	2.6 ± 0.6	2.3 ± 0.1
				g kg^{-1}	
Moisture	4	684.7 ± 12.0	563.5 ± 74.6	674.5 ± 28.7	592.2 ± 11.1
Ash	4	25.5 ± 3.8 a	38.4 ± 4.4 a	46.0 ± 2.1 a	47.3 ± 6.4 b
OM	4	289.7 ± 10.1 a	287.1 ± 25.4 ab	361.9 ± 10.6 b	389.3 ± 61.8 b
Total N	4	7.6 ± 0.5	9.5 ± 1.5	7.1 ± 0.5	7.1 ± 0.2
NH_4/NO_3	4	3.1 ± 1.3	3.2 ± 2.6	4.2 ± 0.2.4	4.7 ± 2.8
P	4	1.1 ± 0.09 a	1.2 ± 0.2 a	0.9 ± 0.06 a	0.8 ± 0.02 ab
K	4	3.9 ± 0.2	4.6 ± 0.6	3.3 ± 0.2	3.7 ± 0.2
Cycle 2					
pH	4	7.6 ± 0.0	7.6 ± 0.1	7.5 ± 0.1	7.4 ± 0.0
C:N ratio	4	14.9 ± 0.6	15.7 ± 1.1	15.8 ± 0.78	20.4 ± 1.7
				dsm^{-1}	
EC	4	2.6 ± 0.5	2.6 ± 0.5	2.6 ± 0.1	2.5 ± 0.1
				g kg^{-1}	
Moisture	4	563.5 ± 74.6	674.5 ± 28.7	507.2 ± 68.8	473.5 ± 83.2
Ash	4	21.1 ± 9.3 a	65.5 ± 3.2 b	62.8 ± 18.8 b	64.6 ± 8.6 b
OM	4	358.1 ± 88.3	415.4 ± 62.0	418.6 ± 42.1	434.6 ± 77.3
Total N	4	13.9 ± 0.8	13.5 ± 1.3	11.0 ± 2.1	10.1 ± 1.0
NH_4/NO_3	4	0.07 ± 0.04	0.1 ± 0.08	0.08 ± 0.03	0.08 ± 0.05
P	4	1.9 ± 0.2	1.5 ± 0.2	0.9 ± 0.3	0.8 ± 0.2
K	4	10.7 ± 3.4	7.9 ± 0.8	6.0 ± 1.4	5.5 ± 0.8

EC, electrical conductivity; OM, organic matter; BC, biochar; Mean ± SE. Numbers with different letters within rows are significantly different at $p < 0.05$.

3.5. Residual Aminopyralid Concentrations

The residual aminopyralid concentrations in the finished composts for the treatments are presented in Table 6. The effects of biochar treatment, year, and year x treatment interaction were significant ($p < 0.05$) for the final residual aminopyralid concentration in the finished composts. The 10 ppb (wet basis) initial aminopyralid concentrations in compost starter mixtures of cycle 1 and 2 were reflected as 33.1–37.3 ppb (dry basis) and (33.8–38.4) ppb (dry basis), respectively. Initial aminopyralid concentrations in the 2–10% BC groups were significantly lower compared to the no BC. The initial aminopyralid levels in the BC treatments of cycle 1 reduced from 47.7 (0% BC) to 100% (10% BC). For cycle 2, aminopyralids in the 0% BC treatment showed slight aggregation compared to the 2.4 to 93.8% reduction in the 2–10% BC treatment. All the treatments with BC reported comparatively higher reduction in aminopyralid concentration compared to the no BC addition.

Table 6. Residual aminopyralid concentration (ppb–dry matter basis) in compost for the treatments after 6-month composting cycles 1 and 2.

		0% BC	2% BC	4% BC	10% BC
	n		Cycle 1		
Initial	4	37.3 ± 0.3 a	34.7 ± 0.9 b	33.1 ± 0.8 b	33.9 ± 0.8 b
Final	4	19.5 ± 2.2 a	15.2 ± 0.7 a	2.5 ± 2.5 b	0.0 ± 0.0 b
% change from initial		47.7	56.2	92.4	100
% difference from 0% BC			8.5	44.7	52.3
			Cycle 2		
Initial	4	38.4 ± 0.5	37.5 ± 2.0	36.1 ± 2.0	33.8 ± 2.6
Final	4	42.1 ± 2.0 a	36.6 ± 4.1 a	22.0 ± 2.8 b	2.1 ± 2.1 c
% change from initial		−0.1	2.4	39.0	93.7
% difference from 0% BC			2.5	39.1	93.8

BC, biochar; Mean ± SE. Numbers with different letters within rows are significantly different at $p < 0.05$.

3.6. After Effects of Residual Aminopyralid in Compost on Tomato Plants

Effect of biochar treatment on growth and injury levels of tomato plants for composting cycle 1 are shown in Table 7. Plant height after the testing period did not differ significantly among the BC treatments but slightly increased with the increase in BC level showing a dose–response relationship. Plant biomass content differed significantly among the treatments with higher biomasses in the treatments with BC compared to the no BC. Plant injury index among the treatments varied between 1.0 (10% BC) and 1.7 (2%), and it was comparatively low in the treatments with higher BC levels.

Table 7. Effect of biochar (BC) treatment on growth and injury levels of tomato plants grown on compost from cycle 1.

Parameter	n	0% BC	2% BC	4% BC	10% BC
Plant height (cm)	4	49.3 ± 1.5	52.0 ± 1.0	53.4 ± 1.6	53.7 ± 1.8
Plant biomass (dry g/plant)	4	4.8 ± 0.3 b	5.2 ± 0.3 ab	6.1 ± 0.3 a	5.9 ± 0.3 a
Mean plant injury index	4	1.6 ± 0.1 a	1.7 ± 0.1 a	1.3 ± 0.1 b	1.0 ± 0.1 b

BC, biochar; Mean ± SE. Numbers with different letters within rows are significantly different at $p < 0.05$.

4. Discussion

Optimization of composting requires initial substrate conditions that facilitate the composting processes. The control of composting parameters such as porosity, nutrient content, C/N ratio, temperature, pH, moisture, and oxygen supply influence composting optimization and determine the optimal condition for microbial development and organic matter degradation [47–50]. The low C/N ratio (13.6–18.7) and excessive moisture content (861–879 g kg^{-1}) of fresh dairy manure (Table 1) was not conducive for an optimal composting process. The chemical and physical properties of sawdust, biochar (not provided), and mineral N supplementation complemented the high moisture content and low C/N ratio of the dairy manure to reach the desired levels. Sawdust, biochar, and mineral N amendment increased the mean C/N ratios of dairy manure from 13.6–18.7 to 27–28 in cycle 1 and to 36–43 in cycle 2 (Table 2) and reached (cycle 1) or exceed (cycle 2) the 25–35 optimum range [51]. Further, sawdust and biochar addition reduced the moisture content of dairy manure from 861–879 g kg^{-1} to 697–739 g kg^{-1} in the compost starter mixtures (Table 2). However, the moisture level in compost starter mixtures exceeded 500–600 g kg^{-1} optimum [45]. A pH of 5.5–8.0 in the compost starter mixture is considered optimum for good microbial activity during composting [48,49]. All compost starter mixtures had slightly higher pH than the optimal highest pH of 8.0, and the values ranged between 8.3 and 8.9 (Table 2). However, the pH of compost starter mixtures was consistent with 8.2–8.8 pH of the same compost starter mixture composted in the windrow experiment reported [52].

Temperature is one of the most important parameters of composting. The composting process transforms fresh organic matter into a bio stable product with a specific heat generation pattern that is highlighted by mesophilic and thermophilic heating [53]. The complete temperature profiles during the two composting cycles (Figures 1 and 2) indicated the occurrence of microbial activity and composting process. Without more frequent early temperature measurements, the presence of typical early mesophilic stage was not evident in the temperature profiles, but it was slightly visible in cycle 2. Although the staging of the typical thermophilic phase was evident in the temperature profiles, the peak temperatures of compost did not exceed the typical 400C. The greater porosity and simulation of microbial activity by the addition of BC for composting showed increasing temperature in compost rapidly [54], reduced the time taken to enter the thermophilic phase, and increased the composting temperatures [55]. However, in this experiment, BC addition did not exert considerable effects on the level of temperature development and the temperature profile.

There was an identical pattern of temperature evolution in the treatments, but the self-heating phase started late by 32nd d in cycle 1 and 56th d in cycle 2. The late commencement of the self-heating phase deviated from the typical 10-day period for full-scale composting [52]. The temperature evolution within the compost corresponded to the natural self-heating of the organic mixture and proved the existence of a thermophilic phase in all reactors. The peak temperatures achieved by composts in all the treatments did not reach the typical >55 °C [52] to meet with the pathogen reduction guideline [48]. The maximum temperatures evolved during thermophilic stages were suboptimal and remained around 40 °C. The deviation from typical peak temperature and the temperature profile could be due to the fact that the low mass of organic matter (14.2 $\pm$ 0.5 kg) involved in this experiment for composting may not be large enough to produce high heat generation and does not result in the thermal inertia of a full-scale system. In addition, the relatively low bulk density and high free air space of compost starter mixtures induced by saw dust and biochar addition may also have contributed high heat loss to lower the level of temperature development during composting.

In contrast to the typical slow and gradual decline in temperature of full-scale composting [56], a rapid decrease in thermophilic temperatures is common within small reactors [57]. The same scenario of rapid decrease in thermophilic temperatures within reactors was noted in this experiment. The identical temperature profiles did not support the effects of BC addition on temperature development in composts. The lower mass of OM involved in this small-scale composting experiment could explain the deviation from the expected temperature developments within composts. Composting degrades OM and the degradation of OM during composting can be estimated as a loss of DM, OM, or organic C [58,59]. During the composting process of cycle 2, the initial total dry weight, total OM, and total OC masses decreased considerably due to the loss of moisture and volatile solids (VSs). The reduction in VS (100- ash content) during composting was supported by the elevated ash contents in the final compost products (Tables 2 and 5). Consistent with [58,59], the loss of DM and OM both showed similar trends among the treatments.

Biochar addition indicated negative effects on the reduction in DM and OM masses with a decreasing trend from 0% BC to 10% BC levels. Biochar provides additional large porosity to dairy manure/saw dust mixture; thus, it is expected that BC addition facilitate more efficient composting. However, BC contains a high amount of aromatic carbon [60], which is more recalcitrant for decomposition than the lignin in saw dust [61]. As such, we suggest that high recalcitrant aromatic carbon in the treatments with BC might have contributed to the relatively lower decompositions of DM and OM compared to the control treatment with no BC. The negative effect of BC on the decomposition of DM and OM was more evident in the 10% BC rate with the lowest mass DM and OM losses (Table 4). The effect of the addition of C by BC [62] and the resulting low degradation of OM was reflected by higher OM contents in the treatments with BC compared to the control treatment (Table 5). Lashermes et al. (2012) [63] reported 40% DM loss while composting a mixture of sewage sludge and green wastes in small composters, whereas our results for composting

dairy manure mixture with no BC in a small composter situation showed much higher loss of DM than [63]. The difference in DM losses noted between the two small-scale experiments could have been attributed to the composition of two compost starter mixtures. However, the total DM loss in the 0% BC treatment was within the range of 44–72% total DM loss reported for dairy manure and sawdust mixture in full-scale composting [52]. The 56% total OM loss observed in the 0% BC treatment was lower than the 67% OM loss found for composting cattle manure under windrow operation [64], but it was comparable to the 46% OM loss found for composting green waste in similar small- scale composters [63].

Nutrient content in compost is an important consideration from the fertility standpoint. The mineralization of OM during composting and concentration of minerals by the loss of OM increased N [65], P, and K [59] mass in the composts. Results from the 0% BC treatment showed a % mass increase (from the initial total mass) of N (4.3%), P (26.5%), and K (25%) comparable to the levels (7–38% N, 14–39% P, and 1–38% K) reported for composting the same mixtures in the windrow system [52]. However, the mean % mass increase in total N and P in the control treatment was considerably lower than the 42–52% N and 40–101% P reported for composting similar starter mixtures in a small heap setting [59]. The difference in % N and P mass increase between the two experiments could be due to the different masses of materials involved and the effect of mass on composting process. Since no leaching took place from the drum reactors, we presume that mineralization of OM during composting and the corresponding loss of OM increased N, P, and K concentrations in the final compost products (Table 5). The increase in N in the treatments with BC could be attributed to the positive effect of BC on sorption of NH_4+ and reducing N losses especially originating from volatilization [66]. Composts with NH_4+/NO_3− ratio < 1.0 [67] or 0.5–3.0 [68] is considered matured. Having NH_4/NO_3 between 0.07 and 0.1 composts from all the treatments of cycle 2 appeared more matured than the composts from cycle 1 with slightly higher NH_4/NO_3 values (3.1–4.7) to be classified as matured (<3.0).

The pH is an important property for plant growth. For most plants, the optimum pH is between 6.5 and 7.0 [55]. The pH determines the mobility of heavy metals; the higher the pH, the lower the solubility of metals [69], and the safer the material from the viewpoint of heavy metal contaminants. Initially, all the treatments had similar pH values that varied between 8.0 and 8.8 (Table 2). Michel et al. (2004) [52] reported that pH in the dairy manure + saw dust composted in windrows decreased pH by <0.5 units to reach 7.7–8.6 final pH in the stabilized composts. Data from the 0% BC treatment of this experiment showed that during composting, pH reduced by 0.7 to 1 unit to a final pH of 7.6–7.8. Several studies on different manure types composted with BC have shown increased pH in the compost, relative to the material without BC addition [70,71]. There are also studies in which the addition of BC to compost substrate did not cause any significant change in pH, compared to the substrate with no BC addition, before and after the completion of the process [55,72]. Results from this experiment agreed with [55,72] and showed no significant difference in pH between the treatments with and without BC.

Composting results in low levels of EC that indicate the high maturity of compost due to less soluble and more stable compounds of high molecular mass [73]. In this experiment, composting dairy manure in small reactors reduced the initial EC values from 2.8–6.9 to 1.9–2.8 dsm^{-1} in the final composts and showed the attainment of maturity. The sorption of soluble organic matter containing various functional groups by biochar contributed to an increase in cation exchange capacity (CEC) and sorption capacity of biochar [74,75]. In addition, electrostatic interaction between metallic ions and the charged biochar surface and complexation or ionic exchange between ionizable protons on the surface of biochar and metallic ions may also have a significant contribution to the biochar's ability to sorb metals [74,76]. However, the higher EC values expected for the treatments with BC were not evident in this experiment. Irrespective of BC level, all the treatments showed similar EC values in the final compost products. The final EC values noted here in this experiment were within the 1.9–3.5 dsm^{-1} range defined for safe seedling growth [36].

The relatively high initial C/N ratios (27.5–36.9) of the control treatment (Table 2) reflected the lignocellulosic nature of the composting substrates. The addition of BC to compost substrates slightly increased the C/N ratio of the compost starter mixtures with peak values in the two cycles ranging from 29.8 to 43.3. In the course of composting, irrespective of BC level, mineralization of substrates or increase in total N taking place after C degradation decreased C/N ratios [55,77,78] from 27.2–29.8 to 19.2–25.7 in cycle 1 and from 36.9–43.3 to 14.9–20 in cycle 2. General compost that is deemed to be mature has C/N < 21 [79], and composts from all the treatments showed C/N within that range. Due to high stability of C in BC, for compost with BC, the value of C/N can be higher than 21 in spite of the compost attaining maturity [79]. However, only the 10% BC treatment from cycle 1 showed C/N exceeding 21. Consistent with [79], the treatments with BC had higher C/N ratios than the control treatment with no BC (Table 5).

The higher C/N ratio in the compost with BC can be explained by the presence of C resistant to degradation added through the BC [80] and to the reduced mineralization of substances in composts with BC [62]. However, some studies have shown no significant difference in the C/N ratio between the compost containing BC and without BC [79]. Our results for 2% and 4% BC rates as well showed no difference in the C/N ratio compared to the control treatment (0% BC) [79]. The C/N ratio is among the certain values that change in the course of composting and is important to estimate the condition of composts. Composts with C/N < 21 or C/N > 21 (for BC amended) are considered to have attained the status of stable/mature [79]. Based on the C/N ratios, compost from all the treatments of this experiment showed attainment of adequate maturity during composting in the two cycles. The C/N, along with other indicators such as EC and NH_4/NH_3, suggests the attainment of stable/mature status by composts where the microbiological processes have slowed down or nearly ceased. In this regard, the uniform abundance of 16S rRNA genes (total bacterial count) among the treatments (Table 5) would provide microbiological evidence for the attainment of stable/mature status by the compost.

In general, composting degrades most herbicides biologically [31,32]. Composting decreased aminopyralid concentration in the treatment with no BC in cycle 1 by 47.7% but aggregated concentration slightly (−0.1%) in cycle 2 (Table 6). Reduction in clopyralid by composting of grass clippings (0.5 m^3)) for a one-year period has been previously reported [33]. In this experiment, clopyralid levels decreased from 32 to 1.4 ppm by 95.6% compared to the 47% decrease in the 0% BC treatment of this experiment. The difference in reduction could be explained by the nature of compost starter mixtures (grass clipping vs. dairy manure) and the initial levels of clopyralid (32 vs. 10 ppb). Aggregation of herbicide due to higher degradation of organic matter that concentrates herbicides in compost has been previously reported [31]. The higher DM loss noted in the 0% BC treatment (Table 4) could explain the aggregation of aminopyralid in cycle 2. Crop residues burn/charcoal/biochar has been shown to adsorb herbicides and reduce their bioavailability [23,81,82]. Further, aminopyralid contains an amine substituent and aromatic amines that are known to sorb strongly to organic matter via electrostatic ion attraction, covalent amine linkages, and organic matter encapsulation [83]. The treatments with BC had higher levels of OM in the finished composts (Table 5) mainly due to the low amount of OM loss (Table 4). Thus, it is expected that BC addition to the dairy manure would enhance the capacity to adsorb aminopyralid. Accordingly, treatments with BC showed higher reduction in aminopyralid (56.2–100% in cycle 1 and 2.4–93.7% in cycle 2) compared to the composting without BC (0–47.7%). Comparable to ours, results from [84] showed that 64–73% of clopyralid (100 ppm) mixed with different organic substrates decomposed within the first 30 days.

During the two composting cycles, the addition of 2, 4, and 10% BC contributed 8.4–12.1%, 44.5–48.7%, and 52.3–100% as much higher reduction in aminopyralid concentration, respectively. The reduction levels were 2.5–93.8% higher than the levels of reduction observed in the control treatment. Further, it was noted that aminopyralids were not detected (<1 ppb) in the samples from higher BC levels. Two samples from 4% BC

treatment, all the samples from 10% BC treatment in cycle 1, and three samples from 10% BC treatment in cycle 2 were noted to have undetectable levels (>1 ppb) of aminopyralids. Biochar addition for dairy manure composting has been found effective for managing residual aminopyralid concentrations. The positive effect of BC to reduce aminopyralid levels in compost could be attributed to the adsorption capacity of BC and positive priming effect of biochar to stimulate the growth of microbial population and activity [24,85], especially during the active phases of composting that promotes higher biodegradation of aminopyralid.

Biological methods for estimating the degree of maturity of composts are based on tests for phytotoxicity. Plant growth tests with combination of germination are the two common biological methods for testing phytotoxicity of composts. Germination tests provide an instant picture of phytotoxicity, whereas growing tests will be affected by continuing changes in the stability or maturity of the compost tested. Effect of biochar treatment on growth and injury levels of tomato plants are shown in Table 7. There were no leaf deformations noted in the 10% BC levels, but there were leaf deformations in the leaves of tomato plants from all other treatments. The intensity of injury to tomato plants measured on the 1–4 scale was significantly lower in the treatments with 4% and 10% BC rates compared to the lower rates (0% and 2%). Addition of BC decreasing plant injury levels in bioassay tests was previously reported [86] for tomato seedlings grown on soil environment where the herbicidal degradation was similar to composting [32]. The height of the plant measured from the root collar to the top of the tomato plant did not differ significantly among the BC treatments, but tomato plants raised on media prepared with compost from higher BC rates grew slightly taller than the plants grown on compost media of low BC rates. The lowest mean plant height was measured in the 0% BC treatment, and the highest was noted in the 10% BC treatment. The auxinic effect of low residual aminopyralid concentrations in the higher BC treatments could explain the slightly higher heights observed for the plants from the treatments with higher BC levels. As with plant height, the dry weight of aboveground phytomass increased with the increase in BC level. The highest above ground phytomass of 5.9–6.1 g was observed in the 4% and 10% BC treatments. Plants from the control treatment reported the lowest plant biomass of 4.8 g. These results confirm the fact that increasing aminopyralid concentration in the media has a growing negative effect on the formation of above ground phytomass of tomato plants [86]. Accordingly, because of low residual aminopyralid levels, tomato plants grown on compost mixtures with higher BC levels, especially with the 4% and 10% levels, reported higher above ground phytomass compared to the plants grown on compost from low BC levels that carried higher levels of aminopyralids. Despite several compost maturity indicators indicating the attainment of maturity, a treatment that carries compost from uncontaminated dairy manure would have been helpful to isolate the magnitude of potential plant damage by other factors resulting from incomplete composting or immature compost.

5. Conclusions

This study examined the chemical and biological characteristics of compost, residual aminopyralid concentrations, and after-effect injuries on tomato plants after composting dairy manure + saw dust mixture with 0%, 2%, 4%, and 10% biochar in 140 L rotatable plastic drum compost reactors for two 6 m cycles. Composting dairy manure with sow dust and biochar in a small reactor setting for 6 months showed C/N and NH_4/NO_3 ratios and electrical conductivity levels to be defined as achieving maturity. Addition of biochar affected negatively the degradation of dry matter and organic matter but exerted a positive effect to concentrate total N, P, & K in compost. Irrespective of the biochar level, composting reduced residual aminopyralid concentrations in the dairy manure with intense reduction by the higher biochar rates (4% and 10%). The compost produced with higher biochar rates also caused mild aftereffect injuries on test plants and promoted plant growth compared to the no and low biochar addition. Establishment of a long term experiment and an upscaled

study in a small heap setting would be beneficial to better understand the effect of biochar on composing processes and altering residual aminopyralid levels in manure compost at a common operational scale.

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References

1. Seefeldt, S.S.; Boydston, R.A.; Kaspari, P.N.; Zhang, M.; Carr, E.; Smeenk, J.; Barnes, D.L. Aminopyralid residue impacts on potatoes and weeds. *Am. J. Potato Res.* **2013**, *90*, 239–244. [CrossRef]
2. Reimer, J. Complicated Composting: Persistent Pyridine Carboxylic Acid Herbicides. 2013. Available online: https://vtechworks.lib.vt.edu/server/api/core/bitstreams/3fdb8840-2900-483b-a493-c92a79f1e812/content (accessed on 8 November 2023).
3. Tremblay, L.A.; Gielen, G.; Northcott, G.L. *Organic Materials Guidelines–Organic Contaminants Review*; Centre for Integrated Biowaste Research: Hamilton, New Zealand, 2016; Volume 23.
4. Rynk, R. Dealing with herbicide residues in compost. *BioCycle* **2000**, *41*, 42.
5. Bezdicek, D.; Fauci, M.; Caldwell, D.; Finch, R.; Lang, J. Persistent herbicides in compost. *BioCycle* **2001**, *42*, 25.
6. Carrithers, V.; Burch, P.; Kline, W.; Masters, R.; Nelson, J.; Halstvedt, M.; Troth, J.; Breuninger, J. Aminopyralid: A new reduced risk active ingredient for control of broadleaf invasive and noxious weeds. In Proceedings of the Western Society of Weed Science 2005, Vancuver, BC, Canada, 8–10 March 2005; pp. 59–60.
7. USEPA. Conventional Reduced Risk Pesticide Program. Available online: https://www.epa.gov/pesticide-registration/conventional-reduced-risk-pesticide-program (accessed on 3 July 2023).
8. Senseman, S.A. *Herbicide Handbook*; Weed Science Society of America: Lawrence, KS, USA, 2007; Volume 458.
9. Hagood, E.S.; Hipkins, P.L.; Hipkins, P.A. Pyridine Herbicide Carryover: Causes and Precautions. 2012. Available online: https://vtechworks.lib.vt.edu/server/api/core/bitstreams/eb4ab09f-1310-40cb-a652-ced9281c6309/content (accessed on 10 November 2023).
10. Shaner, D. *Herbicide Handbook*, 10th ed.; Weed Science Society of America: Lawrence, KS, USA, 2014; Volume 13, pp. 177–178.
11. EFSA, G.O. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). *EFSA J.* **2013**, *11*, 3295.
12. Abe, Y.; Tamura, K.i.; Seike, N. Change of clopyralid concentration in recycled beef cattle compost. *Anim. Sci. J.* **2021**, *92*, e13568. [CrossRef] [PubMed]
13. USEPA. Pesticides-Fact Sheet for Aminopyralid. Available online: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-005100_10-Aug-05.pdf (accessed on 3 July 2023).
14. Davis, J.; Johnson, S.; Jennings, K. *Herbicide Carryover in Hay, Manure, Compost, and Grass Clippings*; North Carolina Cooperative Extension Service, North Carolina State University: Raleigh, NC, USA, 2010; AG-727W.
15. Boutin, C.; Rogers, C.A. Pattern of sensitivity of plant species to various herbicides—An analysis with two databases. *Ecotoxicology* **2000**, *9*, 255–272. [CrossRef]
16. Grossmann, K. Mode of action of auxin herbicides: A new ending to a long, drawn out story. *Trends Plant Sci.* **2000**, *5*, 506–508. [CrossRef] [PubMed]
17. Smith, J.L.; Collins, H.P. Management of organisms and their processes in soils. In *Soil Microbiology, Ecology and Biochemistry*; Elsevier: Amsterdam, The Netherlands, 2007; pp. 471–502.

18. Keener, H.; Elwell, D.; Reid, G.; Michel, F. Composting non-separated dairy manure-theoretical limits and practical experience. In *Animal, Agricultural and Food Processing Wastes, Proceedings of the Eighth International Symposium, Des Moines, IA, USA, 9–11 October 2000*; American Society of Agricultural Engineers: St. Joseph, MI, USA, 2000; pp. 615–623.
19. Awasthi, M.K.; Selvam, A.; Lai, K.M.; Wong, J.W. Critical evaluation of post-consumption food waste composting employing thermophilic bacterial consortium. *Bioresour. Technol.* **2017**, *245*, 665–672. [CrossRef] [PubMed]
20. Young, B.J.; Rizzo, P.F.; Riera, N.I.; Della Torre, V.; López, V.A.; Molina, C.D.; Fernández, F.E.; Crespo, D.C.; Barrena, R.; Komilis, D. Development of phytotoxicity indexes and their correlation with ecotoxicological, stability and physicochemical parameters during passive composting of poultry manure. *Waste Manag.* **2016**, *54*, 101–109. [CrossRef] [PubMed]
21. Miner Jr, F.D.; Koenig, R.T.; Miller, B.E. The influence of bulking material type and volume on in-house composting in high-rise, caged layer facilities. *Compos. Sci. Util.* **2001**, *9*, 50–59. [CrossRef]
22. Iqbal, M.K.; Shafiq, T.; Ahmed, K. Characterization of bulking agents and its effects on physical properties of compost. *Bioresour. Technol.* **2010**, *101*, 1913–1919. [CrossRef] [PubMed]
23. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Vithanage, M.; Lee, S.S.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2014**, *99*, 19–33. [CrossRef] [PubMed]
24. Steiner, C.; Melear, N.; Harris, K.; Das, K. Biochar as bulking agent for poultry litter composting. *Carbon Manag.* **2011**, *2*, 227–230. [CrossRef]
25. Guo, X.-X.; Liu, H.-T.; Zhang, J. The role of biochar in organic waste composting and soil improvement: A review. *Waste Manag.* **2020**, *102*, 884–899. [CrossRef] [PubMed]
26. Li, D.; Zhao, R.; Peng, X.; Ma, Z.; Zhao, Y.; Gong, T.; Sun, M.; Jiao, Y.; Yang, T.; Xi, B. Biochar-related studies from 1999 to 2018: A bibliometrics-based review. *Environ. Sci. Pollut. Res.* **2020**, *27*, 2898–2908. [CrossRef] [PubMed]
27. Inyang, M.; Dickenson, E. The potential role of biochar in the removal of organic and microbial contaminants from potable and reuse water: A review. *Chemosphere* **2015**, *134*, 232–240. [CrossRef]
28. Zhang, X.; Gao, B.; Zheng, Y.; Hu, X.; Creamer, A.E.; Annable, M.D.; Li, Y. Biochar for volatile organic compound (VOC) removal: Sorption performance and governing mechanisms. *Bioresour Technol* **2017**, *245*, 606–614. [CrossRef] [PubMed]
29. Sun, K.; Gao, B.; Ro, K.S.; Novak, J.M.; Wang, Z.; Herbert, S.; Xing, B. Assessment of herbicide sorption by biochars and organic matter associated with soil and sediment. *Environ. Pollut.* **2012**, *163*, 167–173. [CrossRef] [PubMed]
30. Le, M.N.T.; Takahi, M.; Maruyama, K.; Kurisaki, A.; Ohnuma, K. Cardiac differentiation at an initial low density of human-induced pluripotent stem cells. *Vitr. Cell. Dev. Biol. Anim.* **2018**, *54*, 513–522. [CrossRef]
31. Buyuksonmez, F.; Rynk, R.; Hess, T.F.; Bechinski, E. Occurrence, Degradation and Fate of Pesticides During Composting. *Compos. Sci. Util.* **2000**, *8*, 61–81. [CrossRef]
32. Büyüksönmez, F.; Rynk, R.; Hess, T.F.; Bechinski, E. Occurrence, degradation and fate of pesticides during composting: Part I: Composting, pesticides, and pesticide degradation. *Compos. Sci. Util.* **1999**, *7*, 66–82. [CrossRef]
33. Vandervoort, C.; Zabik, M.; Branham, B.; Lickfeldt, D. Fate of selected pesticides applied to turfgrass: Effect of composting on residues. *Bull. Environ. Contam. Toxicol.* **1997**, *58*, 38–45. [CrossRef] [PubMed]
34. Ortiz-Hernández, M.L.; Sánchez-Salinas, E.; Dantán-González, E.; Castrejón-Godínez, M.L. Pesticide biodegradation: Mechanisms, genetics and strategies to enhance the process. *Biodegrad. -Life Sci.* **2013**, *10*, 251–287.
35. Van Eerd, L.L.; Hoagland, R.E.; Zablotowicz, R.M.; Hall, J.C. Pesticide metabolism in plants and microorganisms. *Weed Sci.* **2003**, *51*, 472–495. [CrossRef]
36. Brinton, W.; Evans, E.; Blewett, T. Reliability of bioassay tests to indicate herbicide residues in compost of varying salinity and herbicide levels. *Compos. Sci. Util.* **2006**, *14*, 244–251. [CrossRef]
37. Blewett, T.C.; Roberts, D.W.; Brinton, W.F. Phytotoxicity factors and herbicide contamination in relation to compost quality management practices. *Renew. Agric. Food Syst.* **2005**, *20*, 67–72. [CrossRef]
38. Alpaslan, M.; Gunes, A. Interactive effects of boron and salinity stress on the growth, membrane permeability and mineral composition of tomato and cucumber plants. *Plant Soil* **2001**, *236*, 123–128. [CrossRef]
39. Fauci, M.; Bezdicek, D.; Caldwell, D.; Finch, R. Development of plant bioassay to detect herbicide contamination of compost at or below practical analytical detection limits. *Bull. Environ. Contam. Toxicol.* **2002**, *68*, 79–85. [CrossRef]
40. Mason, I.; Milke, M. Physical modelling of the composting environment: A review. Part 1: Reactor systems. *Waste Manag.* **2005**, *25*, 481–500. [CrossRef]
41. Ryckeboer, J.; Mergaert, J.; Vaes, K.; Klammer, S.; De Clercq, D.; Coosemans, J.; Insam, H.; Swings, J. A survey of bacteria and fungi occurring during composting and self-heating processes. *Ann. Microbiol.* **2003**, *53*, 349–410.
42. Sanz, E.; Prats, D.; Rodriguez, M.; Camacho, A. Effect of temperature and organic nutrients on the biodegradation of linear alkylbenzene sulfonate (LAS) during the composting of anaerobically digested sludge from a wastewater treatment plant. *Waste Manag.* **2006**, *26*, 1237–1245. [CrossRef]
43. Haug, R. *The Practical Handbook of Compost Engineering*; Routledge: London, UK, 2018.
44. Rynk, R.; Van de Kamp, M.; Willson, G.B.; Singley, M.E.; Richard, T.L.; Kolega, J.J.; Gouin, F.R.; Laliberty, L.; Kay, D.; Murphy, D. On-Farm Composting Handbook. 1992. Northeast Regional Agricultural Engineering Services Publication 54, Ithaca, NY, USA. Available online: https://campus.extension.org/pluginfile.php/48384/course/section/7167/NRAES%20FarmCompost%20manual%201992.pdf (accessed on 3 July 2023).

45. Gajalakshmi, S.; Abbasi, S. Solid waste management by composting: State of the art. *Crit. Rev. Environ. Sci. Technol.* **2008**, *38*, 311–400. [CrossRef]
46. Sparks, D.L.; Page, A.L.; Helmke, P.A.; Loeppert, R.H. *Methods of Soil Analysis, Part 3: Chemical Methods*; John Wiley & Sons: Hoboken, NJ, USA, 2020; Volume 14.
47. Agnew, J.; Leonard, J. The physical properties of compost. *Compos. Sci. Util.* **2003**, *11*, 238–264. [CrossRef]
48. De Bertoldi, M.d.; Vallini, G.; Pera, A. The biology of composting: A review. *Waste Manag. Res.* **1983**, *1*, 157–176. [CrossRef]
49. Miller, F.C. Composting as a process based on the control of ecologically selective factors. In *Soil Microbial Ecology: Applications in Agricultural and Environmental Management*; Marcel Dekker Inc.: New City, NY, USA, 1992; pp. 515–544.
50. Richard, T.L.; Hamelers, H.; Veeken, A.; Silva, T. Moisture relationships in composting processes. *Compos. Sci. Util.* **2002**, *10*, 286–302. [CrossRef]
51. Bishop, P.L.; Godfrey, C. Nitrogen transformations during sludge composting. *Biocycle* **1983**, *24*, 34–39.
52. Michel Jr, F.C.; Pecchia, J.A.; Rigot, J.; Keener, H.M. Mass and nutrient losses during the composting of dairy manure amended with sawdust or straw. *Compos. Sci. Util.* **2004**, *12*, 323–334. [CrossRef]
53. Van Der Wurff, A.W.; Fuchs, J.; Raviv, M.; Termorshuizen, A. *Handbook for Composting and Compost Use in Organic Horticulture*; BioGreenhouse: Humanes de Madrid, Spain, 2016.
54. Li, R.; Wang, Q.; Zhang, Z.; Zhang, G.; Li, Z.; Wang, L.; Zheng, J. Nutrient transformation during aerobic composting of pig manure with biochar prepared at different temperatures. *Environ. Technol.* **2015**, *36*, 815–826. [CrossRef]
55. Zhang, J.; Chen, G.; Sun, H.; Zhou, S.; Zou, G. Straw biochar hastens organic matter degradation and produces nutrient-rich compost. *Bioresour. Technol.* **2016**, *200*, 876–883. [CrossRef]
56. Petiot, C.; De Guardia, A. Composting in a laboratory reactor: A review. *Compos. Sci. Util.* **2004**, *12*, 69–79. [CrossRef]
57. Petric, I.; Šestan, A.; Šestan, I. Influence of initial moisture content on the composting of poultry manure with wheat straw. *Biosyst. Eng.* **2009**, *104*, 125–134. [CrossRef]
58. Garrison, M.V.; Richard, T.L.; Tiquia, S.M.; Honeyman, M.S. Nutrient losses from unlined bedded swine hoop structures and an associated windrow composting site. In Proceedings of the 2001 ASAE Annual Meeting, American Society of Agricultural and Biological Engineers, Sacramento, CA, USA, 29 July– 1 August 2001; p. 1.
59. Parkinson, R.; Gibbs, P.; Burchett, S.; Misselbrook, T. Effect of turning regime and seasonal weather conditions on nitrogen and phosphorus losses during aerobic composting of cattle manure. *Bioresour. Technol.* **2004**, *91*, 171–178. [CrossRef]
60. Lehmann, J.; Amonette, J.E.; Roberts, K.; Hillel, D.; Rosenzweig, C. Role of biochar in mitigation of climate change. In *Handbook of Climate Change and Agroecosystems: Impacts, Adaptation, and Mitigation*; Imperial College Press: London, UK, 2010; pp. 343–363.
61. Preston, C.M.; Schmidt, M.W. Black (pyrogenic) carbon: A synthesis of current knowledge and uncertainties with special consideration of boreal regions. *Biogeosciences* **2006**, *3*, 397–420. [CrossRef]
62. Jindo, K.; Sánchez-Monedero, M.A.; Hernández, T.; García, C.; Furukawa, T.; Matsumoto, K.; Sonoki, T.; Bastida, F. Biochar influences the microbial community structure during manure composting with agricultural wastes. *Sci. Total Environ.* **2012**, *416*, 476–481. [CrossRef]
63. Lashermes, G.; Barriuso, E.; Le Villio-Poitrenaud, M.; Houot, S. Composting in small laboratory pilots: Performance and reproducibility. *Waste Manag.* **2012**, *32*, 271–277. [CrossRef]
64. Bernal, M.P.; Alburquerque, J.; Moral, R. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* **2009**, *100*, 5444–5453. [CrossRef]
65. Bernal, M.P.; Navarro, A.F.; Roig, A.; Cegarra, J.; Garcia, D. Carbon and nitrogen transformation during composting of sweet sorghum bagasse. *Biol. Fertil. Soils* **1996**, *22*, 141–148. [CrossRef]
66. Hua, L.; Wu, W.; Liu, Y.; McBride, M.B.; Chen, Y. Reduction of nitrogen loss and Cu and Zn mobility during sludge composting with bamboo charcoal amendment. *Environ. Sci. Pollut. Res.* **2009**, *16*, 1–9. [CrossRef]
67. Ko, H.J.; Kim, K.Y.; Kim, H.T.; Kim, C.N.; Umeda, M. Evaluation of maturity parameters and heavy metal contents in composts made from animal manure. *Waste Manag.* **2008**, *28*, 813–820. [CrossRef]
68. Thompson, W.; Leege, P.; Millner, P.; Watson, M. *TMECC: Test Methods for the Examination of Composting and Compost*; US Composting Council Research and Education Foundation and the United States Department of Agriculture: Washington, DC, USA, 2002.
69. Abd El-Azeem, S.A.; Ahmad, M.; Usman, A.R.; Kim, K.-R.; Oh, S.-E.; Lee, S.S.; Ok, Y.S. Changes of biochemical properties and heavy metal bioavailability in soil treated with natural liming materials. *Environ. Earth Sci.* **2013**, *70*, 3411–3420. [CrossRef]
70. Chen, Y.-X.; Huang, X.-D.; Han, Z.-Y.; Huang, X.; Hu, B.; Shi, D.-Z.; Wu, W.-X. Effects of bamboo charcoal and bamboo vinegar on nitrogen conservation and heavy metals immobility during pig manure composting. *Chemosphere* **2010**, *78*, 1177–1181. [CrossRef]
71. Czekała, W.; Malińska, K.; Cáceres, R.; Janczak, D.; Dach, J.; Lewicki, A. Co-composting of poultry manure mixtures amended with biochar–The effect of biochar on temperature and C-CO2 emission. *Bioresour. Technol.* **2016**, *200*, 921–927. [CrossRef] [PubMed]
72. Zhang, J.; Lü, F.; Shao, L.; He, P. The use of biochar-amended composting to improve the humification and degradation of sewage sludge. *Bioresour. Technol.* **2014**, *168*, 252–258. [CrossRef]
73. Zbytniewski, R.; Buszewski, B. Characterization of natural organic matter (NOM) derived from sewage sludge compost. Part 1: Chemical and spectroscopic properties. *Bioresour. Technol.* **2005**, *96*, 471–478. [CrossRef]

74. Borchard, N.; Prost, K.; Kautz, T.; Moeller, A.; Siemens, J. Sorption of copper (II) and sulphate to different biochars before and after composting with farmyard manure. *Eur. J. Soil Sci.* **2012**, *63*, 399–409. [CrossRef]
75. Zeng, G.; Wu, H.; Liang, J.; Guo, S.; Huang, L.; Xu, P.; Liu, Y.; Yuan, Y.; He, X.; He, Y. Efficiency of biochar and compost (or composting) combined amendments for reducing Cd, Cu, Zn and Pb bioavailability, mobility and ecological risk in wetland soil. *RSC Adv.* **2015**, *5*, 34541–34548. [CrossRef]
76. Vithanage, M.; Herath, I.; Joseph, S.; Bundschuh, J.; Bolan, N.; Ok, Y.S.; Kirkham, M.; Rinklebe, J. Interaction of arsenic with biochar in soil and water: A critical review. *Carbon* **2017**, *113*, 219–230. [CrossRef]
77. Benitez, E.; Sainz, H.; Nogales, R. Hydrolytic enzyme activities of extracted humic substances during the vermicomposting of a lignocellulosic olive waste. *Bioresour. Technol.* **2005**, *96*, 785–790. [CrossRef] [PubMed]
78. Jindo, K.; Sonoki, T.; Matsumoto, K.; Canellas, L.; Roig, A.; Sanchez-Monedero, M.A. Influence of biochar addition on the humic substances of composting manures. *Waste Manag.* **2016**, *49*, 545–552. [CrossRef]
79. Khan, N.; Clark, I.; Sánchez-Monedero, M.A.; Shea, S.; Meier, S.; Bolan, N. Maturity indices in co-composting of chicken manure and sawdust with biochar. *Bioresour. Technol.* **2014**, *168*, 245–251. [CrossRef]
80. Wang, C.; Lu, H.; Dong, D.; Deng, H.; Strong, P.; Wang, H.; Wu, W. Insight into the effects of biochar on manure composting: Evidence supporting the relationship between N2O emission and denitrifying community. *Environ. Sci. Technol.* **2013**, *47*, 7341–7349. [CrossRef] [PubMed]
81. Semple, K.T.; Riding, M.J.; McAllister, L.E.; Sopena-Vazquez, F.; Bending, G.D. Impact of black carbon on the bioaccessibility of organic contaminants in soil. *J. Hazard. Mater.* **2013**, *261*, 808–816. [CrossRef] [PubMed]
82. Soni, N.; Leon, R.G.; Erickson, J.E.; Ferrell, J.A.; Silveira, M.L. Biochar decreases atrazine and pendimethalin preemergence herbicidal activity. *Weed Technol.* **2015**, *29*, 359–366. [CrossRef]
83. Godskesen, B.; Holm, P.E.; Jacobsen, O.S.; Jacobsen, C.S. Aging of triazine amine in soils demonstrated through sorption, desorption, and bioavailability measurements. *Environ. Toxicol. Chem. Int. J.* **2005**, *24*, 510–516. [CrossRef] [PubMed]
84. Lubytė, J.D.; Antanaitis, Š.; Antanaitis, A. Decomposition of chlopiralid, tribenuron-methyl and pendimethalin in different organic substrates. *Ekologija* **2007**, *53*, 77.
85. Zhang, L.; Sun, X. Changes in physical, chemical, and microbiological properties during the two-stage co-composting of green waste with spent mushroom compost and biochar. *Bioresour. Technol.* **2014**, *171*, 274–284. [CrossRef]
86. Soukupová, M.; Koudela, M. Impacts of Aminopyralid on Tomato Seedlings. *Horticulturae* **2023**, *9*, 456. [CrossRef]