agriculture

Article

Spectroscopic Investigation on the Effects of Biochar and Soluble Phosphorus on Grass Clipping Vermicomposting

Etelvino Henrique Novotny [1,*], Fabiano de Carvalho Balieiro [1], Ruben Auccaise [2], Vinícius de Melo Benites [1] and Heitor Luiz da Costa Coutinho [1,†]

[1] Embrapa Solos, Rua Jardim Botânico, 1024, Rio de Janeiro 22460-000, Brazil; fabiano.balieiro@embrapa.br (F.d.C.B.); vinicius.benites@embrapa.br (V.d.M.B.)
[2] Departamento de Física, Universidade Estadual de Ponta Grossa, Av. General Carlos Cavalcanti 4748, Ponta Grossa 84030-900, Brazil; raestrada@uepg.br
* Correspondence: etelvino.novotny@embrapa.br
† In memorian.

check for updates

Citation: Novotny, E.H.; Balieiro, F.d.C.; Auccaise, R.; Benites, V.d.M.; Coutinho, H.L.d.C. Spectroscopic Investigation on the Effects of Biochar and Soluble Phosphorus on Grass Clipping Vermicomposting. *Agriculture* **2022**, *12*, 1011. https://doi.org/10.3390/agriculture12071011

Academic Editor: Borbála Biró

Received: 25 April 2022
Accepted: 7 July 2022
Published: 13 July 2022

Abstract: Seeking to evaluate the hypothesis that biochar optimises the composting and vermicomposting processes as well as their product quality, we carried out field and greenhouse experiments. Four grass clipping composting treatments (only grass, grass + single superphosphate (SSP), grass + biochar and grass + SSP + biochar) were evaluated. At the end of the maturation period (150 days), the composts were submitted to vermicomposting (Eisenia fetida earthworm) for an additional 90 days. Ordinary fine charcoal was selected due to its low cost (a by-product of charcoal production) and great availability; this is important since the obtained product presents low commercial value. A greater maturity of the organic matter (humification) was observed in the vermicompost treatments compared with the compost-only treatments. The addition of phosphate significantly reduced the pH (from 6.7 to 4.8), doubled the electrical conductivity and inhibited biological activity, resulting in less than 2% of the number of earthworms found in the treatment without phosphate. The addition of soluble phosphate inhibited the humification process, resulting in a less-stable compound with the preservation of labile structures, primarily cellulose. The P species found corroborate these findings because the pyrophosphate conversion from SSP in the absence of biochar may explain the strong acidification and increased electric conductivity. Biochar appears to prevent this conversion, thus mitigating the deleterious effects of SSP and favouring the formation of organic P species from SSP (78.5% of P in organic form with biochar compared to only 12.8% in the treatments without biochar). In short, biochar decreases pyrophosphate formation from SSP, avoiding acidification and salinity; therefore, biochar improves the whole composting and vermicomposting process and product quality. Vermicompost with SSP and biochar should be tested as a soil conditioner on account of its greater proportion of stabilized C and organic P.

Keywords: ^{13}C nuclear magnetic resonance; ^{31}P nuclear magnetic resonance; charcoal; *Eisenia fetida*; pyrogenic carbon

1. Introduction

Biochar is a C-rich product distinct from charcoal because it is produced for soil application purposes in order to improve its quality (organic matter stability, cation exchange capacity (CEC), soil fertility, water retention capacity, porosity and biological activity are commonly increased), prevent nutrient leaching, improve C storage or purify the soil from pollutants [1–3]. The uses of and studies regarding this pyrolyzed biomass for agriculture have increased in recent decades, in part due to recognition of the role of carbonised biomass in the high fertility of *terra preta de indios* soils, which are anthropic soils that had a high input of carbonised biomass during the pre-Colombian period [4–6]. Although a number of studies have investigated the effects of different pyrolyzed residues on plant productivity and chemical and physical soil properties [7–11], little is known about *biochar* interactions with soil microbiota and fauna or with the native microorganisms from the raw materials used in composting [8,12–14].

Composting is a technique in which microbial activity during organic material decay is optimised and the material is transformed into either a fertiliser or soil conditioner. Vermicomposting, on the other hand, is a process by which earthworms aid in recycling and increase the velocity of organic matter stabilisation through residue homogenisation. The degradation of cellulose and inoculation of other organisms are stimulated by these macrofauna representatives, and humification tends to increase in the presence of these organisms [15–17].

Although earthworms occur in most environments, these organisms are sensitive to soil management practices [18,19], trace metals (mainly Cd) [20,21] and carbon polyaromatic-containing substances [21,22]; thus, they can be used as quality indicators for soil or different organic substrates [21,23,24]. Fertilisers can affect the abundance and density of earthworms in the soil, although limited studies have investigated the effect of soluble sources, including phosphorus (P), on these soil organisms. Sarathchandra et al. [25] performed field studies and did not observe changes in the populations of *Lumbricus rubellus* or *Aporrectodea caliginosa* in either the adult or young forms after three years of single superphosphate (SSP) and rock phosphate application in North Carolina. However, laboratory studies have shown that direct contact with SSP can be lethal to the Californian red earthworm (*Eisenia fetida*), which has led to studies recommending the monitoring of these animal populations after applications of inorganic and soluble sources of P to the field [26].

In order to find a way to minimise the external input (fertilisers and soil conditioner) needed to maintain the extensive grasslands at the Rio de Janeiro International Airport in Brazil, which produces approximately 2 Mg of grass clippings daily, and also to reduce the disposal cost of this low-density raw material and hence reduce the environmental impact of the airport, the potential use of grass clippings for compost was proposed [27,28]. Therefore, this study aimed to evaluate the impact of using soluble phosphate and biochar on the establishment and development of the Californian red earthworm *E. fetida* in compost and vermicompost that included grass clippings from the Rio de Janeiro International Airport as well as evaluate the final products using ^{13}C and ^{31}P solid-state nuclear magnetic resonance spectroscopy. We hypothesized that the introduction of P in the composting process would increase the nutritional status of the compost/vermicompost, since P is a limiting element to plant growth in tropical oxidized soils, and that biochar would improve the composting and vermicomposting processes as well as the soil conditions for grass growth in the airport field area (not tested).

2. Materials and Methods

2.1. Composting Site and Origin of Raw Material

The experiment was conducted in a covered hangar of the Rio de Janeiro/Galeão International Airport (−22.804114°; −43.229841°), which is located in Rio de Janeiro, Brazil. Composting was performed using grass clippings obtained by mowing the airport's islands, i.e., the unused grassy areas between taxiways, between runways or between a taxiway and a runway. The clippings were ground to homogenise the particle size. The chemical composition of the residue (mean ± standard deviation, n=20) was as follows [28]: C = 415 ± 2 g kg^{-1}; N = 11 ± 2 g kg^{-1}; K = 10.7 ± 0.7 g kg^{-1}; Ca = 4.2 ± 0.7 g kg^{-1}; Mg = 1.3 ± 0.1 g kg^{-1}; P = 0.5 ± 0.1 g kg^{-1}; and C/N ratio = 47 ± 7.5. At the beginning of the experiment, the average moisture and density of the grass clippings were 70–80% and ~80 kg m^{-3}, respectively.

2.2. Composting of Grass Clippings

Four treatments arranged in a full factorial design (2^2: two levels of phosphate and of *biochar* in grass clipping composting) were tested: grass (G); grass + biochar (GB); grass + phosphate (GP); and grass + biochar + phosphate (GBP). The phosphate used was single superphosphate (SSP) with 8.7% P, mainly in the form of monocalcium phosphate (). Previous composting was performed in 2.5 m^3 piles. The charcoal used in the piles was the residue of vegetable charcoal production (*Eucalyptus* sp., conventional carbonisation at 450 °C for six hours). This residue was a fine-grained charcoal, with a grain size usually smaller than 5 mm. The used sample exhibited non-uniform and small grain sizes, with only 10% of the mass retained on a 4-mm sieve. The quantities of each input used to prepare the compost piles are presented in Table 1.

Table 1. Quantities (kg) of each material/input used in the composting piles (2.5 m^3).

Treatments	Grass (kg)	Biochar (kg)	Phosphate (kg)
Grass	200	-	-
Grass + biochar	160	143	-
Grass + phosphate	200	-	25.6
Grass + phosphate + biochar	160	143	25.6

At the beginning of the process, the piles were manually turned every two days. After 30 days, the piles were turned every 10 days. After 120 days of composting, all of the piles were ground to 5 cm, and the piles were turned twice a week for 30 days.

After 150 days of composting, six sub-samples of each pile were collected and transferred to plastic boxes for vermicomposting.

2.3. Vermicomposting

Vermicomposting was performed for 90 days using a completely randomised experimental design with six replicates. Twenty adult *E. fetida* were added to 10 L of the different composts and placed in 20 L plastic boxes. *E. fetida* earthworms were chosen because they exhibit high prolificacy, precociousness, survival rates and adaptability to captivity [4]. The experimental design was a 2 × 4 full factorial design (with and without earthworms × 4 former treatments, see Section 2.2) with 6 replicates and a total of 48 worm boxes.

Compost moisture was kept constant with frequent wettings. After 90 days, the earthworms from each box were counted.

2.4. Physical, Chemical and Spectroscopy Characterisation

Sub-samples from each box were dried at 45 °C for 48 h and ground, and the C and N concentrations were determined using a PerkinElmer 2400 CHNS (Waltham, MA, USA) elemental analyser. The pH and electrical conductivity (EC) of the different composts were determined as follows: approximately 15 g of compost was weighed in lidded plastic containers and then 85 mL of distilled water was added. The samples were stirred using a horizontal agitator for 30 min at 40 rpm and left to stand for 20 min, after which the EC and pH were measured.

For spectroscopic characterisation, sub-samples of each treatment were freeze-dried and ground in liquid N_2. Solid-state ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectra were obtained with a Varian Inova (11.74 T) spectrometer at ^{13}C, ^{31}P and ^{1}H frequencies of 125.7, 202.5 and 500.0 MHz, respectively. For this, a T3NB HXY with a 4-mm probe was utilised to detect the ^{13}C and ^{31}P nuclei and the rotors were spun using dry air at 15 kHz. All experiments were carried out at room temperature.

Two NMR pulse procedures were applied: variable amplitude cross-polarisation (CP) for ^{13}C and direct polarisation (DP) for ^{31}P.

In the ^{13}C CP-MAS experiment an optimised recycle delay (d1) of 500 ms was used; the ^{1}H 90-pulse was set to 3 μs, the contact time value to 1 ms and the acquisition time to 15 ms. High-power two-pulse phase modulation (TPPM) ^{1}H decoupling at 70 kHz was employed. The cross-polarisation time was selected after variable contact time experiments and the recycle delays were selected to be five times longer than the longest spin–lattice relaxation time (T_1), as determined by inversion recovery experiments.

In the ^{31}P DP-MAS experiment, the recycle delay was 10 min (after inversion-recovery experiments), the ^{31}P 90-pulse was set to 3 µs and the acquisition time to 15 ms.

2.5. Data Analysis

The data from the vermicomposting experiment (pH, EC and number of earthworms) were subjected to a multivariate analysis of variance (MANOVA), while the data from composting and further vermicomposting were analysed using repeated measures MANOVA. The normality and homoscedasticity of the residuals were tested and, when necessary, appropriate data transformation (log for C and C/N ratio and square root for the number of earthworms) was applied. When a statistically significant effect was detected, the means were compared using Duncan's test at $p < 0.05$. The software Statistica 7.1 [29] was used for all statistical analyses.

The multivariate analysis of the spectral data was performed using the software Unscrambler 10.4 (CAMO, Norway). Principal component analysis (PCA) was carried out using the full ^{13}C NMR spectra obtained, after area normalisation and mean-centring of the data. To aid in the analyses of the ^{31}P NMR results, the multivariate curve resolution (MCR) procedure was carried out. The basic goals of MCR are: the determination of the number of components co-existing in the chemical system; the extraction of the pure spectra of the components (qualitative analysis); and the extraction of the concentration profiles of these components (quantitative analysis). This analysis was preceded by PCA to estimate the number of components in the mixture. After this, the rotation of the PC was calculated without orthogonality constraints (in this way, it would have infinite solutions). To solve this, new constraints were adopted (e.g., non-negative concentrations and/or non-negative spectra). In this way, when the goals of MCR are achieved, it is possible to unravel the "true" underlying sources of data variation, after which the results with physical meaning are easily interpretable [18].

3. Results

3.1. Earthworm Reproduction with Different Substrates

The application of only phosphate drastically inhibited earthworm reproduction (Figure 1a). However, the interaction between the main factors of phosphate and biochar was statistically significant ($p = 2.6 \times 10^{-6}$), with this detrimental effect mitigated by biochar, resulting in significantly larger populations than the GP treatment but still lower populations than the treatments without phosphate (G and GB).

3.2. Chemical and Spectroscopy Analysis of the Composts and Vermicomposts

The addition of phosphate fertiliser increased the medium's acidity and the EC of the vermicompost; however, biochar decreased these effects significantly (Figure 1b,c). Biochar prevented the pronounced acidification caused by phosphate; however, the pH was still lower than that of the treatments without phosphate (significant interaction, $p = 2.0 \times 10^{-6}$). Meanwhile, for EC just the main factors (phosphate and biochar) were statistically significant ($p = 5.5 \times 10^{-6}$ and 9.9×10^{-4}, respectively), without significant interaction; therefore, the biochar decreased the EC, fully mitigating the deleterious effect of phosphate application on the vermicompost EC.

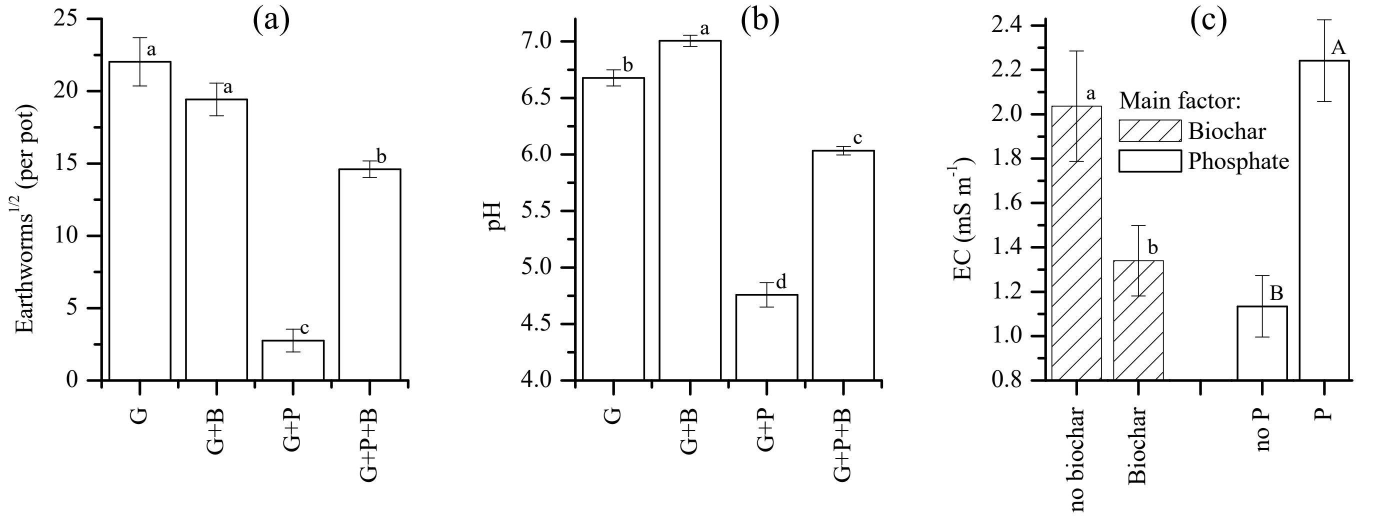

Figure 1. Square root of earthworm number after inoculation for 90 days in different composted substrates (**a**); pH (**b**) and electrical conductivity (EC) (**c**) of the vermicompost. G: grass clippings; G + B: grass + biochar; G + P: grass + phosphate; and G + B + P: grass + biochar + phosphate. Columns with the same lower-case letters (for earthworms, pH and biochar factor for EC) and upper-case letters (for P factor for EC) indicate no statistical difference at $p < 0.05$ using Duncan test. Vertical bars denote standard errors.

The introduction of biochar increased C concentrations in all substrates (Figure 2a), and no significant effect was observed for phosphate or vermicomposting. Concerning N concentration and C/N ratio, the interaction of phosphate x biochar was significant ($p = 2.8 \times 10^{-4}$ and 2.5×10^{-4}, respectively), being that phosphate and biochar decreased N and increased the C/N ratio (Figure 2b), with the strongest effect being from biochar. In addition, further vermicomposting increased the N content and decreased the C/N ratio (Figure 2c), indicating further humification.

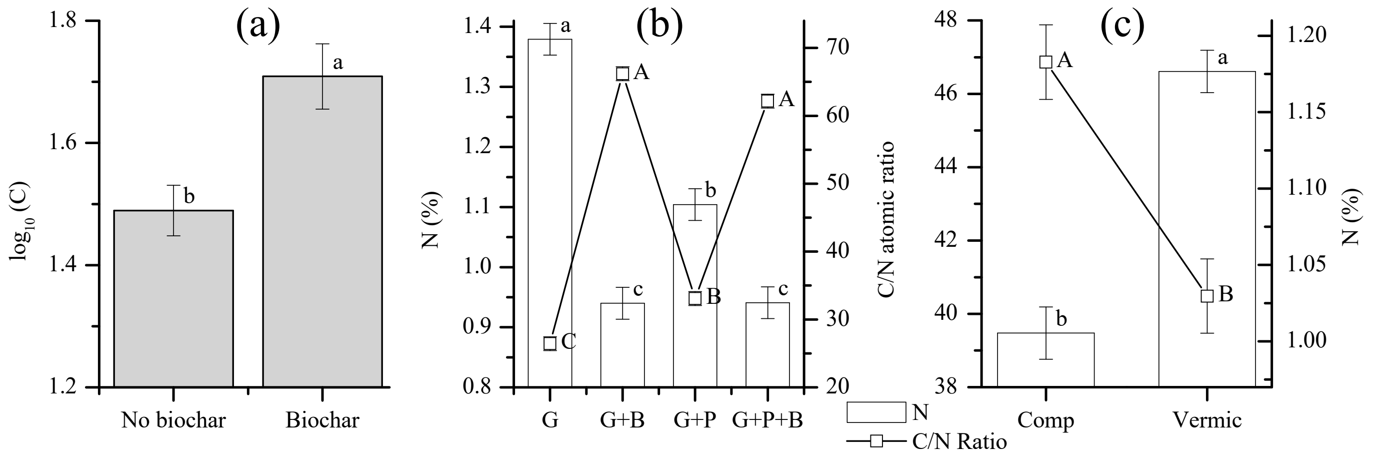

Figure 2. Biochar effect on C concentration (**a**), phosphate and biochar interaction effects on N concentration and on C/N ratio (**b**), and effects of vermicomposting on these variables (**c**). G: grass clippings; G + B: grass + biochar; G + P: grass + phosphate; and G + B + P: grass + biochar + phosphate; Comp: composting; and Vermic: further vermicomposting. Columns with the same lower-case letters (C and N) and symbols with the same upper-case letters (C/N ratio) indicate no statistical difference at $p < 0.05$ using Duncan test. Vertical bars denote standard errors.

The change in humification was confirmed by the ^{13}C and ^{31}P NMR spectra, which are shown below. The C/N ratio increased in all treatments with biochar (Figure 2b), which was also explained by the inclusion of material rich in recalcitrant C and poor in N. Meanwhile, a significant effect of vermicomposting we observed was that it decreased the C/N ratio. The addition of phosphate to the substrates in the absence of biochar resulted in higher C/N ratios in the compost and vermicompost (no interaction), confirming the inhibition of humification by phosphate.

The organic chemical structures detected using ^{13}C NMR (Figure 3) indicate that, following composting, the material still exhibited significant amounts of labile structures, mainly cellulose (O-alkyl, with signals at ~65 and 73 ppm and di-O-alkyl, with signal at ~105 ppm). The relative contribution of these regions varied between substrates, indicating selective degradation of cellulose (higher biological activity and higher humification) in certain substrates. For the substrates with biochar, a pronounced increase of the C-aryl signal was observed (~125 ppm), which is a typical signal of condensed aromatic rings. These differences were summarised and highlighted using PCA and are discussed in detail below.

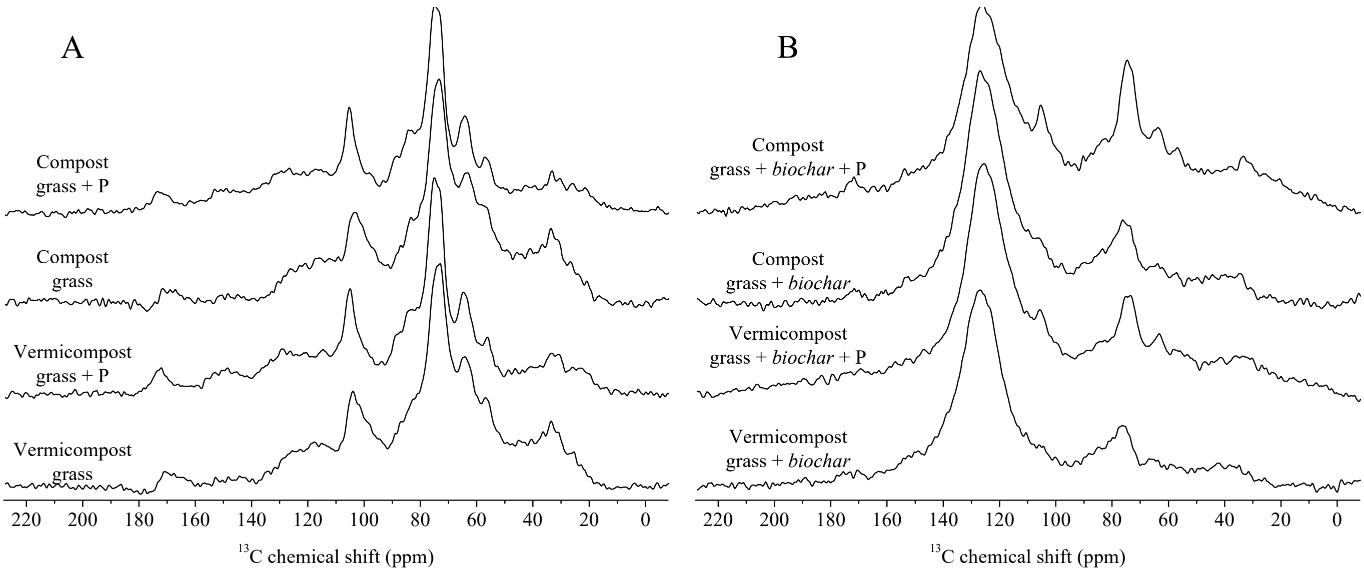

Figure 3. Solid state ^{13}C NMR spectra of the different compost and vermicompost obtained. Without biochar (**A**) and with biochar (**B**).

3.3. Progress of Humification According to Principal Component Analysis (PCA)

Using PCA, we were able to satisfactorily model the data (97% of variance captured) with two principal components (PCs). The first PC (92% of total variance) identified the different substrates exhibiting bipolar loadings (Figure 4A), with positive loadings for the sp^2 C signals of biochar (aryl) and negative for the sp^3 hybridised C of grass cellulose (O-alkyl and di-O-alkyl). The second PC (5% of total variance) was characterised by negative loadings for the signals typical of labile structures (Figure 4A), primarily cellulose, that were partially oxidised to uronic acids (O-alkyl, di-O-alkyl and carboxyl with a signal at ~173 ppm). Therefore, this PC served as an indicator of the progress of humification, since less labile structures indicate the progression of the humification process.

The samples with biochar (GB, VGB, GBP, VGBP) presented the highest scores for PC1 (Figure 4B), confirming the contribution of polycondensed aryl structures towards their ^{13}C NMR spectra.

The samples containing only grass, with and without earthworms, exhibited the highest PC2 scores, indicating a lower proportion of labile structures and more advanced humification (Figure 4B). Treatments with phosphate and without biochar (compost—GP and vermicompost—VGP) exhibited the lowest scores for PC2 (Figure 4B), indicating that phosphate inhibited the advance of humification by suppressing the biological activity of micro- and macro-organisms (after the earthworm population decrease, Figure 1a), resulting in a material rich in labile structures, i.e., partially oxidised cellulose. This finding confirmed the results for the N concentrations and C/N ratios as well as the detrimental effects on earthworms. The samples with biochar (GB, VGB, GBP, VGBP) exhibited intermediate scores (Figure 4B) for this component (uronic acids), indicating that the maturation of the compost and vermicompost were similar for the samples with biochar, regardless of phosphate presence.

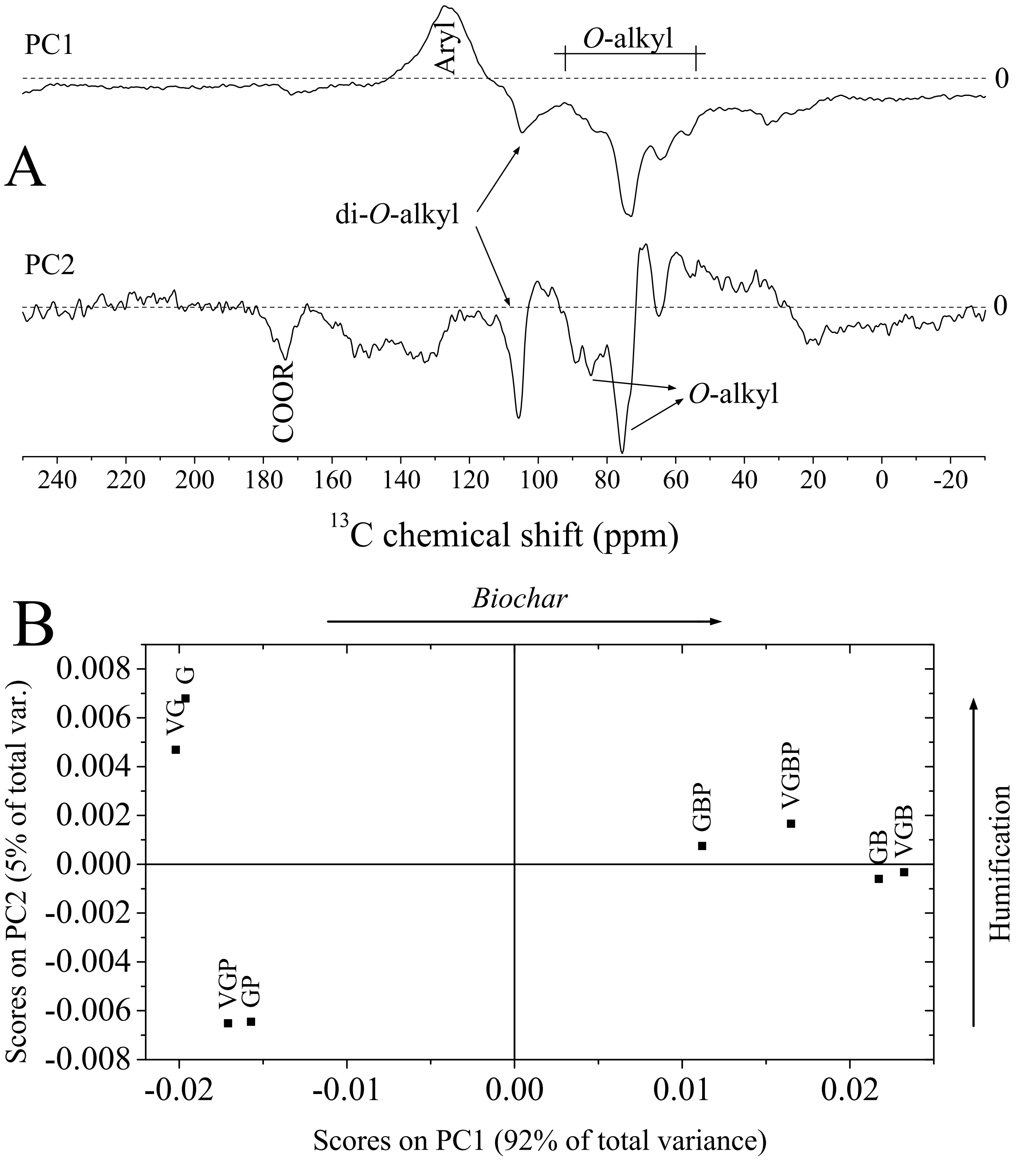

Figure 4. (**A**) PCA loadings obtained from the ^{13}C NMR spectra. (**B**) PCA scores. G: grass compost; VG: grass vermicompost; GP: grass + phosphate compost; VGP: grass + phosphate vermicompost; GBP: grass + biochar + phosphate compost; VGBP: grass + biochar + phosphate vermicompost.

The ^{31}P NMR and its multivariate curve resolution analysis (Figure 5) indicate that, in the absence of biochar, the addition of phosphate to the compost significantly changed the distribution of P species, with a likely predominance of pyrophosphate (with a chemical shift of approximately −1.3 ppm). Moreover, in samples without phosphate or with phosphate and biochar, the predominant form was likely organic (mono and di-ester phosphate, with chemical shifts of approximately 0.96 ppm and 2 ppm, respectively) or mixed with non-hydrolysed monobasic phosphate (chemical shift of 0.96 ppm).

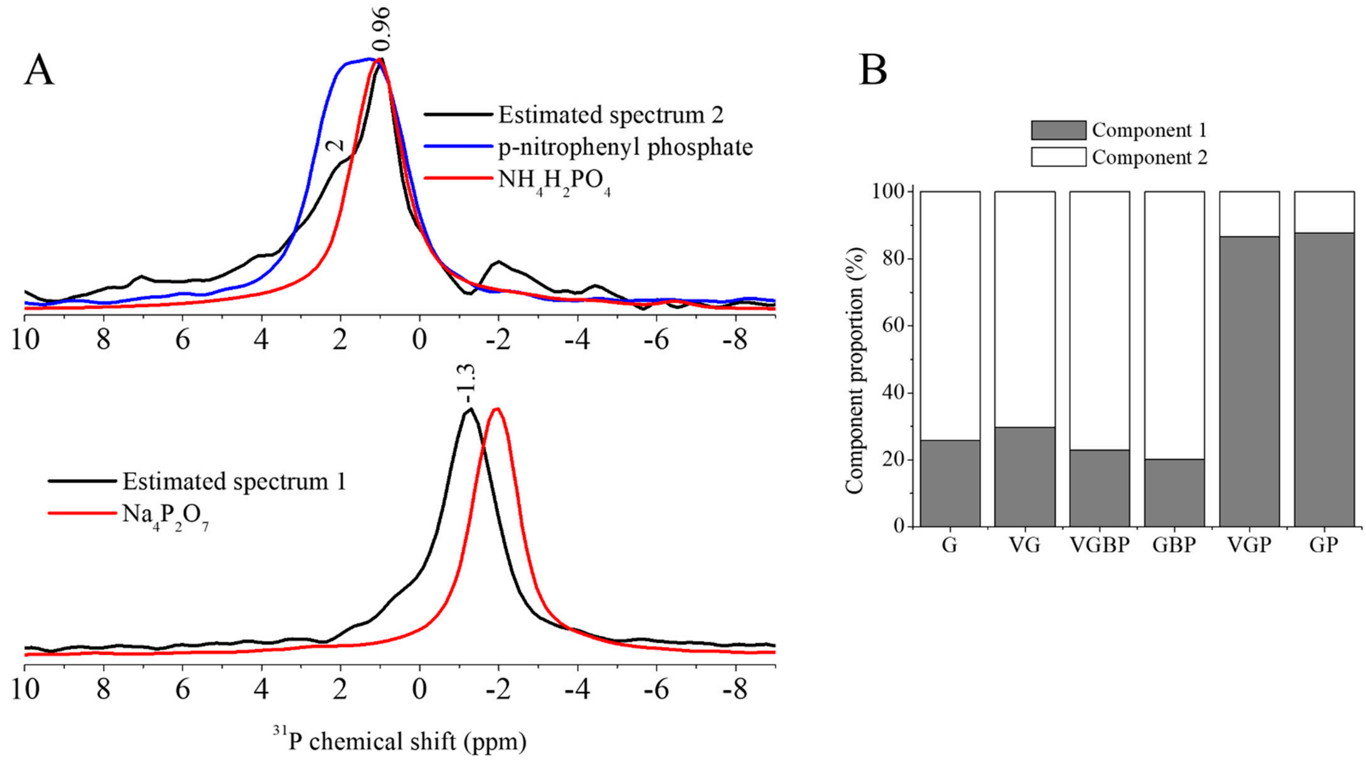

Figure 5. Multivariate curve resolution results obtained from the ^{31}P NMR spectra showing the two component solutions. (**A**): estimated component spectra; discontinued lines show the ^{31}P NMR spectra of ^{31}P standards. (**B**): estimated concentrations. G: grass compost; VG: grass vermicompost; GP: grass + phosphate compost; VGP: grass + phosphate vermicompost; GBP: grass + biochar + phosphate compost; and VGBP: grass + biochar + phosphate vermicompost.

4. Discussion

The grass clipping compost obtained from the International Airport of Rio de Janeiro did not exhibit limitations for composting, which was consistent with many other works [27,28]. We found that grass clippings could also be used for vermicomposting because a statistically significant increase of 400% of the earthworm population was observed during the studied period (Figure 1a). Curry and Schmidt [30] cited, amongst a wide range of organic materials, the presence of grass in the digestive tract of these soil animals, which indicates that grass clipping material can be used to feed earthworms and then in vermicomposting.

However, when SSP was added to the composting substrate, a detrimental effect was observed. The acid reaction from the sulphate present in the phosphate inhibited microbial activity, with the resulting compost exhibiting lower N concentrations, higher C/N ratios (Figure 2b) and higher levels of labile structures (uronic acids—Figure 4). Moreover, the phosphate severely inhibited earthworm reproduction and led to a significant decrease in the earthworm population (i.e., death or escape of the earthworms, Figure 1a). The phosphate dosage (1 kg m^{-3}) used in the present experiment resulted in a significant increase in compost acidity ($pH < 5$) and in the ionic strength of the substrates (Figure 1b,c), which are factors that negatively affect the development and reproduction of earthworms of this genus [31]. This acidification may result from the conversion of applied phosphate into pyrophosphate in the absence of biochar, as detected by ^{31}P NMR (Figure 5). On the other hand, in the absence of phosphate or in the presence of biochar, the predominant forms of P were organic or non-hydrolysed monobasic phosphate, indicating the highest biological activity and incorporation of P into biological tissues.

In addition, the presence of certain metals (Cd, Cu, Ni, As, Cr and Pb) and radionuclides (^{226}Ra, ^{228}Ra and ^{210}Pb) has been reported in phosphate fertilisers commercialised in Brazil [32], albeit in reduced concentrations. However, their solubility may have increased because of the pronounced pH decrease, thus contributing to the detrimental effect on the earthworms. Nevertheless, additional specific studies are required to confirm this hypothesis. It should be noted that in a review on the uptake and accumulation of heavy metals by earthworms, the metal concentration in the soil was found to be a poor predictor of metal concentration in the earthworms' tissues, and pH was considered to be the main factor associated with metal uptake by earthworms [33].

The presence of biochar mitigated these detrimental effects of phosphate. The pyrolysed biomass acquired different properties depending on the pyrolysis temperature and nature of the feedstock [13,34]. Biochar application has been found to promote changes in nutrient availability (usually pH increase, N immobilisation etc.) [19]; interference with organism and plant signalling compounds; xenobiotic detoxification; and refuge availability for pathogens or growth-promoting organisms, etc. These changes are examples of multiple beneficial or detrimental effects (direct and indirect) of biochar on soil quality and plant production [24,35–37].

The litter earthworm is sensitive to high acidity, high salinity and certain toxic elements, and the treatments with biochar exhibited increased pH and decreased EC, similar or close to the values from the treatments without phosphate. These characteristics may have contributed to the mitigation of detrimental effects of phosphate on the earthworm population and also towards microbiological activity, as indicated by the humification of the biomass (Figure 4).

The ^{31}P NMR spectra and their MCR analysis (Figure 5) show that, in the absence of biochar, the addition of phosphate to the compost significantly changed the distribution of P species, with a likely predominance of pyrophosphate. Moreover, in samples without phosphate or with phosphate and biochar, the predominant form was likely organic (mono and di-ester phosphate) or mixed with monobasic phosphate. This conversion of applied phosphate into pyrophosphate in the absence of biochar may explain the strong acidification of the medium as well as the increase in EC. Biochar was able to prevent this conversion and favoured the formation of P organic species, which may have been caused by the highest microbial activity in the presence of biochar as indicated by the highest compost humification (Figure 3).

Vermicomposting increased the N concentration of the tested substrates (Figure 2c), probably due to the labile organic matter evolving to CO_2 and resulting in a relative enrichment in N.

Biochar addition to the substrates led to lower N concentrations in the compost and vermicompost (Figure 2b), which may have been caused by a dilution effect related to the inclusion of material with high recalcitrant C concentrations and low N concentrations.

A decrease in the C/N atomic ratios was observed after vermicomposting (Figure 2c), indicating a further humification process promoted by the earthworms. The relative increase of N concentrations likely occurred because the raw materials were N-poor and cellulose degradation (CO_2 emission) was favoured by the presence of earthworms; we confirmed this using the ^{13}C NMR spectra (Figures 3 and 4).

5. Conclusions

A detrimental effect of SSP on macro- and micro-biota was observed with a drastic decrease in earthworm population and lower compost and vermicompost humification.

This detrimental effect of SSP was likely caused by acidic conditions resulting from pyrophosphate formation and high salinity, inhibiting humification. This was indicated by the presence of materials with increased labile structures, low N concentrations and high C/N ratios. However, biochar mitigated this negative effect during composting by maintaining microbial activity, which was indicated by higher humification, and during vermicomposting, by mitigating the detrimental effect of SSP on the earthworm population. Biochar probably reduces the deleterious acidity and salinity induced by single superphosphate by decreasing pyrophosphate formation, avoiding acidification and salinity.

SSP alone is not recommended for the composting and vermicomposting of grass clippings, but in combination with biochar it should be tested as a soil conditioner, on account of its greater proportion of stabilized C (from grass clippings and pyrogen) and organic P.

Author Contributions: Conceptualisation (E.H.N.; F.d.C.B.; V.d.M.B. and H.L.d.C.C.); methodology (E.H.N.; F.d.C.B.; R.A. and V.d.M.B.); investigation (E.H.N.; F.d.C.B.; R.A. and V.d.M.B.); formal analysis (E.H.N.); visualisation (E.H.N.); writing—original draft, review and editing (E.H.N.; R.A.; F.d.C.B. and H.L.d.C.C.); supervision and funding acquisition (E.H.N.; F.d.C.B.; V.d.M.B. and H.L.d.C.C.); project administration (F.d.C.B.). All authors have read and agreed to the published version of the manuscript.

Funding: Funding was provided by: the Funding Authority for Studies and Projects (Financiadora de Estudos e Projetos—FINEP) grant project "Production of organic fertilisers and substrates from residues of grass maintenance in urban areas"; the National Counsel of Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq) for Novotny's and Balieiro's research fellowships (309391/2020-2 and 307434/2020-6, respectively); the Brazilian Federal Agency for Support and Evaluation of Graduate Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES) for Auccaise's post-doctoral research fellowships (PNPD Nº 03020/09-6); and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for Novotny's research fellowship (E-26/202.874/2018).

Institutional Review Board Statement: Not applicable since the work does not involve humans or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors wish to thank the Nuclear Magnetic Resonance Laboratory of the Brazilian Centre of Physics Research (Laboratório de Ressonânica Magnética Nuclear do Centro Brasileiro de Pesquisas Físicas). The authors wish to thank the Embrapa Soils trainees (scholarship granted - PIBIC/Embrapa Solos): João Paulo Moura Barata, Carolina Araújo de Queiroz Costa and Natália Evaristo Belo do Santos, as well as the airport workers who helped conduct the experiments at the airport and at Embrapa Soils. I thank Adriana Aquino from Embrapa Agrobiology for the donation of earthworms and recommendations for the management of the experiment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Glaser, B.; Balashov, E.; Haumaier, L.; Guggenberger, G.; Zech, W. Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Org. Geochem.* **2000**, *31*, 669–678. [CrossRef]
2. Cunha, T.J.F.; Madari, B.E.; Benites, V.M.; Canellas, L.P.; Novotny, E.H.; Moutta, R.O. Chemical fractionation of organic matter and humic acid characterization in anthropogenic dark earth soils of the Brazilian Amazon region. *Acta Amazon.* **2007**, *37*, 91–98. [CrossRef]
3. Novotny, E.H.; Hayes, M.H.B.; Madari, B.; Bonagamba, T.J.; Azevedo, E.R.; Souza, A.A.; Song, G.; Nogueira, C.M.; Mangrich, A.S. Lessons from the Terra Preta de Índios of the Amazon region for the utilisation of charcoal for soil amendment. *J. Braz. Chem. Soc.* **2009**, *20*, 1003–1010. [CrossRef]
4. Novotny, E.H.; Maia, C.M.B.F.; Carvalho, M.T.M.; Madari, B.E. Biochar: Pyrogenic carbon for agricultural use—A critical review. *Rev. Bras. Cienc. Solo* **2015**, *39*, 321–344. [CrossRef]

5. Glaser, B.; Haumaier, L.; Guggenberger, G.; Zech, W. The 'Terra Preta' phenomenon: A model for sustainable agriculture in the humid tropics. *Naturwissenschaften* **2001**, *88*, 37–41. [CrossRef]
6. Novotny, E.H.; Azevedo, E.R.; Bonagamba, T.J.; Cunha, T.J.F.; Madari, B.E.; Benites, V.M.; Hayes, M.H.B. Studies of the compositions of humic acids from Amazonian dark earth soils. *Environ. Sci. Technol.* **2007**, *41*, 400–405. [CrossRef]
7. Steiner, C.; Glaser, B.; Teixeira, W.G.; Lehmann, J.; Blum, W.E.H.; Zech, W. Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferralsol amended with compost and charcoal. *J. Plant Nutr. Soil Sci.* **2008**, *171*, 893–899. [CrossRef]
8. Noguera, D.; Rondón, M.; Laossi, K.; Hoyos, V.; Lavelle, P.; Cruz de Carvalho, M.H. Contrasted effect of biochar and earthworms on rice growth and resource allocation in different soils. *Soil Biol. Biochem.* **2010**, *42*, 1017–1027. [CrossRef]
9. Ogawa, M.; Okimori, Y. Pioneering works in biochar research, Japan. *Aust. J. Soil Res.* **2010**, *48*, 489–500. [CrossRef]
10. Kookana, R.S.; Sarmah, A.K.; van Zwieten, L.; Krull, E.; Singh, B. Biochar application to soil: Agronomic and environ-mental benefits and unintended consequences. *Adv. Agron.* **2011**, *112*, 103–143. [CrossRef]
11. Petter, F.A.; Madari, B.E.; Silva, M.A.S.D.; Carneiro, M.A.C.; Carvalho, M.T.D.M.; Marimon Júnior, B.H.; Pacheco, L.P. Soil fertility and upland rice yield after biochar application in the Cerrado. *Pesqui. Agropecu. Bras.* **2012**, *47*, 699–706. [CrossRef]
12. Saito, M.; Marumoto, T. Inoculation with arbuscular mycorrhizal fungi: The status quo in Japan and future prospects. *Plant Soi* **2002**, *244*, 273–279. [CrossRef]
13. Sanpa, S.; Imaki, K.; Sumiyoshi, S.; Shibata, A.; Matsumiya, Y.; Kubo, M. Effect of charcoal from woody waste on the soil bacterial biomass and its plant-growth promoting effect. *Wood Carbonization Res.* **2006**, *2*, 37–42.
14. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota—A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [CrossRef]
15. Lavelle, P.; Barros, E.; Blanchart, E.; Brown, G.; Desjardins, T.; Mariani, L. SOM management in the tropics. Why feed the soil macrofauna? *Nutr. Cycl. Agroecos.* **2001**, *61*, 53–61. [CrossRef]
16. Whiston, R.A.; Seal, K.J. The occurrence of cellulases in the earthworm *Eisenia foetida*. *Biol. Wastes* **1988**, *25*, 239–242. [CrossRef]
17. Dores-Silva, P.R.; Landgraf, M.D.; Rezende, M.O.O. Chemical monitoring of vermicomposting from domestic sewage sludge. *Quim. Nova* **2011**, *34*, 956–961. [CrossRef]
18. Aquino, A.M.; Oliveira, A.M.G.; Loureiro, D.C. Integrating composting and vermicomposting on the recycling of domestic organic waste. *Embrapa Agrobiol.-Circ. Técnica* **2005**, *12*, 1–4.
19. Giannopoulos, G.; Pulleman, M.M.; Van Groenigen, J.W. Interactions between residue placement and earthworm ecological strategy affect aggregate turnover and N_2O dynamics in agricultural soil. *Soil Biol. Biochem.* **2010**, *42*, 618–625. [CrossRef]
20. Abdul Rida, A.M.M.; Bouché, M.B. The eradication of an earthworm genus by heavy metals in Southern France. *Appl. Soil Ecol.* **1995**, *2*, 45–52. [CrossRef]
21. Li, M.; Liu, Z.; Xu, Y.; Cui, Y.; Li, D.; Kong, Z. Comparative effects of Cd and Pb on biochemical response and DNA damage in the earthworm *Eisenia fetida* (*Annelida*, *Oligochaeta*). *Chemosphere* **2009**, *74*, 621–625. [CrossRef]
22. Hartenstein, R. Effect of aromatic compounds, humic acids and lignins on growth of the earthworm *Eisenia foetida*. *Soil Biol. Biochem.* **1982**, *14*, 595–599. [CrossRef]
23. Loureiro, S.; Soares, A.M.; Nogueira, A.J. Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. *Environ. Pollut.* **2005**, *138*, 121–131. [CrossRef] [PubMed]
24. International Organization for Standardization. Soil Quality—Avoidance Test for Determining the Quality of Soils and Effects of Chemicals on Behaviour—Part 1: Test with Earthworms (Eisenia fetida and Eisenia andrei), Geneva. 2008. Available online: https://www.sis.se/api/document/preview/910491/ (accessed on 11 December 2019).
25. Sarathchandra, S.U.; Lee, A.; Perrott, K.W.; Rajan, S.S.S.; Oliver, E.H.A.; Gravett, I.M. Effects of phosphate fertilizer applications on microorganisms in pastoral soil. *Aust. J. Soil Res.* **1993**, *31*, 299–309. [CrossRef]
26. Abbiramy, K.S.K.; Ross, P.R.; Paramanandham, J.P. Assessment of acute toxicity of superphosphate to *Eisenia fetida* using paper contact method. *Asian J. Plant Sci. Res.* **2013**, *3*, 112–115.
27. Nakasaki, K.; Hiraoka, S.; Nagata, H. A new operation for producing disease-suppressive compost from grass clippings. *Appl. Environ. Microbiol.* **1998**, *64*, 1015–4020. [CrossRef] [PubMed]
28. Benites, V.M.; Bezerra, F.B.; Mouta, R.O.; Assis, I.R.; Santos, R.C.; Conceição, M. Production of organic fertilisers from composting of waste from the maintenance of grass areas of the Rio de Janeiro International airport. In *Boletim de Pesquisa e Desenvolvimento*; Embrapa-CNPS: Rio de Janero, Brazil, 2004.
29. SAS Institute Inc. *SAS®Visual Statistics 7.1: User's Guide*; SAS Institute Inc.: Cary, NC, USA, 2014.
30. Curry, J.P.; Schmidt, O. The feeding ecology of earthworms—A review. *Pedobiologia* **2006**, *50*, 463–477. [CrossRef]
31. Kaplan, D.L.; Hartenstein, R.; Neuhauser, E.F.; Malecki, M.R. Physicochemical requirements in the environment of the earthworm *Eisenia foetida*. *Soil Biol. Biochem.* **1980**, *12*, 347–352. [CrossRef]
32. Saueia, C.H.R.; Le Bourlegat, F.M.; Mazzilli, B.P.; Fávaro, D.I.T. Availability of metals and radionuclides present in phosphogypsum and phosphate fertilizers used in Brazil. *J. Radioanal. Nucl. Chem.* **2013**, *297*, 189–195. [CrossRef]
33. Nahmani, J.; Hodson, M.E.; Black, S. A review of studies performed to assess metal uptake by earthworms. *Environ. Pollut.* **2007**, *145*, 402–424. [CrossRef]
34. Czimczik, C.I.; Masiello, C.A. Controls on black carbon storage in soils. *Glob. Biogeochem. Cycles* **2007**, *21*, GB3005. [CrossRef]
35. Rondon, M.A.; Lehmann, J.; Ramírez, J.; Hurtado, M. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol. Fert. Soils* **2007**, *43*, 699–708. [CrossRef]

36. Warnock, D.D.; Lehmann, J.; Kuyper, T.W.; Rillig, M.C. Mycorrhizal responses to biochar in soil—Concepts and mechanisms. *Plant Soil* **2007**, *300*, 9–20. [CrossRef]
37. Anderson, C.R.; Condron, L.M.; Clough, T.J.; Fiers, M.; Stewart, A.; Hill, R.A. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* **2011**, *54*, 309–320. [CrossRef]