Effects of Biochar to Excessive Compost-Fertilized Soils on the Nutrient Status

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Abstract: Positive effects of a biochar–compost mix on soil nutrient status in infertile soil have been reported, but the potential effect of biochar amendments in excessive compost-fertilized soils has not been extensively studied. Excessive application of compost can result in the accumulation of nutrients and heavy metals (Cu and Zn). Thus, the objective of this study is to investigate the effect of biochar–excessive compost co-application on soil nutrient status. We hypothesized that biochar co-application could have positive effects on the absorption of excessive nutrients of Cu and Zn. A 371-day laboratory incubation study was conducted to evaluate the effects of the lead tree (Leucaena leucocephala (Lam.) de. Wit) biochar produced at 750 °C on the dynamics of the soil nutrients. Three Taiwan rural soils were selected, including slightly acidic Oxisols (SAO), mildly alkaline Inceptisols (MAI), and slightly acid Inceptisols (SAI). The biochar treatments include control (0%) and 0.5%, 1.0%, and 2.0% (w/w). In each treatment, 5% (w/w) poultry-livestock manure compost was added to test excessive application. The results indicated that the biochar treatments had a significant increase effect on soil pH, total carbon (TC), total nitrogen (TN), C:N ratio, and available K concentration. The effect of biochar on electrical conductivity (EC) and available P, Ca, Mg, Fe, Mn, Cu, Pb, and Zn was insignificant. The effect of biochar, with relatively low application rates (<2% by wt), low surface area, and less surface function group, was eliminated by excessive compost (5% by wt). In addition to carbon sequestration and nitrogen conservation, biochar addition has no effect on the absorption of the excessive nutrients Cu and Zn in three studied soils.

Keywords: biochar; soil nutrient status; sustainable agriculture; excessive compost-fertilized soil; soil type

1. Introduction

Poor fertility of highly weathered soils raises concerns about the sustainability of agriculture and has motivated the development of management practices to restore or improve fertility [1]. Accordingly, the use of biochar has attracted increasing interest as a sustainable means of improving highly weathered or degraded tropical soils [2]. The application of charcoal to degraded agricultural soils has been found to improve soil fertility in the USA [3], but no change was observed in extractable soil nutrients of Aridisols in Australia [4]. Furthermore, soil-extractable P, K, and Fe increased in a Mollisols [5], with a notable increase in soil-extractable P, K, Mg, and Ca in a Mollisols [6], a decrease in P leaching in two Aridisols [7], an improvement of soil fertility and carbon sequestration in Ultisols [8], and significant improvement of soil physicochemical properties in a moderately acidic low-input Nepalese soil [9]. In addition, a more prominent improvement in soil fertility can be achieved by biochar application to the sandy soil having low clay, nutrient, and organic matter contents [10]. Biochar co-application with manure to calcareous soil had several beneficial effects on soil properties [11], including improving the soil water status, which would be considered beneficial in areas where irrigation or rainfall is limited; a positive synergistic increase in soil-extractable Zn content occurred, which could also be beneficial in Zn-deficient calcareous soils. Berek et al. [12] assessed the capacity of biochars in combination...
with composts to enhance the availability of plant nutrients in two highly weathered soils of Hawai’i (Oxisols and Ultisols), and the results suggested that locally produced biochars and composts be used to improve plant nutrient availability in the highly weathered soils.

The application of biochar may provide a new management option with the potential to increase the long-term capacity of soils to retain and recycle nutrients and to reduce nutrient loss in agriculture [13]. Indeed, the biochar amendment has a great potential to enhance soil fertility and crop yield by reducing soil acidity, increasing soil CEC and nutrient retention, and improving plant nutrient availability. The enhancement, however, is dependent on the biochar type, the application rate, and soil type [14]. In three European countries (Italy, Spain, and Belgium), the results of Oldfield et al. [15] indicated that biochar, compost, and biochar–compost blend all resulted in lower environmental impacts (global warming, acidification, and eutrophication) than mineral fertilizer from a systems perspective. Regional differences were found between biochar, compost, and biochar–compost blend, and the biochar-compost blend offered benefits related to available nutrients and sequestered C. Although many studies have reported the positive effects of a biochar–compost mix on soil properties and plant growth [15–19], most were in infertile soil. The enormous soil erosion, nutrient loss, and rapid decomposition of soil organic matter are the major setbacks to Taiwan’s agricultural soils. The recommended doses of organic compost in Taiwan is about 1% to 2% (w/w), according to economic viability. However, some farmers apply excessive organic compost (about 2% to 5% (w/w)) to add more N in intensive cultivation periods for short-term leafy crops (about 30,000 ha) because of the low N mineralization rate [20] and relatively low levels of nutrients compared to complete fertilizer [21], and thus, this results in providing excess N, P, and other nutrients. The inefficient use of nutrients will cause negative environmental impacts [15,21,22]. The effect of biochar–excessive compost co-application on soil nutrient status has been less examined.

The lead tree (or white popinac) (Leucaena leucocephala (Lam.) de. Wit), an exotic plant introduced into Taiwan by the Dutch in 1643, was originally used for paper production and forest establishment, and as a forage resource. However, because its economic value decreased over time, this plant has been left to proliferate by itself. However, the lead tree has a strong ability to compete with other plants. For the purpose of rebuilding original forest communities in Taiwan after the long-term invasion by the lead tree, a lot of lead trees were cut down by local governments. Owing to its diameter of breast height (DBH) mostly less than 10 cm, those cut-down lead trees cannot be processed and utilized further. If those loggings are put in situ, the decomposing loggings will continue to release vast CO₂ into the atmosphere and will lead to a negative impression on the public. On the contrary, if the loggings can be used to produce charcoal (biochar), it is a win–win scenario for reducing carbon dioxide emissions and sequestering much carbon. In addition, several inadequate characteristics, such as poor physical properties, low stabilization degree, or the presence of substances that might eventually be phytotoxic, usually restricted the use of large percentages of composts in growth media [23], as well as into the soil. Furthermore, Fornes et al. [23] summarized and indicated that compost phytotoxicity could have several origins, including high pH and electrical conductivity (EC) due to high salt concentration, which is a characteristic of composts produced from agricultural wastes or from manure; accumulation of phenolic compounds; high concentration of NH₃ and NH₄⁺, which is characteristic of manure composts; high content of heavy metals, mainly Cu and Zn, which is characteristic of sewage sludge and pig slurry composts. In Taiwan, rural soil, the application of excessive compost could result in the accumulation of nutrients and heavy metals (Cu and Zn). The excessive nutrients could be lost due to soil surface erosion and/or soil leaching, and the excessive Cu and Zn could cause crop toxicity and reduce yield. In addition to the beneficial potential of biochar for carbon sequestration [24] and available inorganic N (NO₃⁻-N+NH₄⁺-N) retention [25] in excessive compost-fertilized soil, we hypothesized that biochar co-application could have the positive effects on the absorption of excessive nutrients, Cu and Zn. The influence of biochar co-application would depend on soil type, biochar rate, and incubation time. Thus, a 371-day laboratory incubation study was conducted with the objective of investigating the effect of biochar-excessive compost co-application on soil nutrient status.
2. Materials and Methods

2.1. Soils, Biochar, and Compost

The characterization of the studied soils (15 cm depth), biochar, and poultry-livestock manure compost has been described in previous studies (Table S1) [24–27]. Three studied soils, including Pingchen (Pc) soil (red earth, slightly acidic Oxisols (SAO)), Erhlin (Eh) soil (fluvo-aquic soil, mildly alkaline Inceptisols (MAI)), and Annei (An) soil (fluvo-aquic soil, slightly acid Inceptisols (SAI)) were collected. According to the occupied area in rural land, these three studied soils are the top ten rural soils in Taiwan. The occupied area is 17,734, 21,158, and 12,754 ha for SAO, MAI, and SAI soil, respectively. Soil pH and soil texture of the SAO, MAI, and SAI were pH 6.1 and clay (59% clay), pH 7.5 and clay loam (39% clay), and pH 6.5 and clay loam (34% clay), respectively.

Biochar produced from the stems and branches of the lead tree in an earth kiln was constructed by the Forest Utilization Division, Taiwan Forestry Research Institute, Taipei, Taiwan. The charring for earth kilns typically requires 7–10 days, and the highest temperature was above 750 °C at the end of carbonization. The characteristics of studied biochar were as follows: pH 9.9, EC 0.77 dS m\(^{-1}\), total C 81.1%, total N 7.49 g kg\(^{-1}\), C:N ratio 97:1, total P 0.55 g kg\(^{-1}\), exchangeable K 1.91 cmol(+) kg\(^{-1}\) soil\(^{-1}\), Na 1.26 cmol(+) kg\(^{-1}\) soil\(^{-1}\), Ca 3.62 cmol(+) kg\(^{-1}\) soil\(^{-1}\), and Mg 0.40 cmol(+) kg\(^{-1}\) soil\(^{-1}\), cation exchange capacity (CEC) 5.20 cmol(+) kg\(^{-1}\) soil\(^{-1}\), base saturation (BS) 100%, Mehlich 3 extractable P 96.6 mg kg\(^{-1}\), K 616 mg kg\(^{-1}\), Ca 4.09 g kg\(^{-1}\), Mg 27.8 g kg\(^{-1}\), Cu 0.02 mg kg\(^{-1}\), Pb not detected, and Zn 0.35 mg kg\(^{-1}\). In addition, the O/C, H/C, and O/H molar ratios were 0.185, 0.018, and 10.2, respectively [27], indicating the high aromaticity of the sample. The specific surface area (BET) of the biochar (determined by measuring N\(_2\) adsorption) was 36.6 m\(^2\) g\(^{-1}\), the scanning electron microscopy (SEM) analysis revealed a highly porous surface structure with a high prevalence of macropores (>50 nm) [26]. The Fourier-transform infrared spectroscopy (FTIR) spectrum of the lead tree biochar displayed various low-intensity bands [27], corresponding to the stretching of OH in alcohol and phenol (3600–3200 cm\(^{-1}\)), methyl CH asymmetric -CH\(_3\) (2950 cm\(^{-1}\)), C=O bonds (1750 cm\(^{-1}\)), aliphatic C-H (1470–1455 cm\(^{-1}\)), C-O stretching in the syringyl ring (1329 cm\(^{-1}\)), C-O stretching in the guaiacyl ring (1270 cm\(^{-1}\)), and C-O stretching in the O-CH\(_3\) and C-OH groups (1100 cm\(^{-1}\)). A band at 875 cm\(^{-1}\) could be attributed to the out-of-plane bending of the aromatic C-H, indicating the high aromaticity of the sample, and the presence of carbonate ions (CO\(_3^{2-}\)), as confirmed by the X-ray diffraction (XRD) patterns.

The poultry-livestock manure compost used in this study is the commercial product (organic fertilizer) certified by the government and often used by farmers. The main raw materials (>50%) of the studied compost were poultry manure (mostly chicken) and livestock manure (mostly swine), and the minor raw material was mushroom waste, which was completely decomposed after a composting period of 6 months. The dry matter content was higher than 65%, according to regulations. The basic characteristics of studied compost were pH 8.4, EC 3.79 dS m\(^{-1}\), total C 23.3%, total N 22.6 g kg\(^{-1}\), total P 10.2 g kg\(^{-1}\), exchangeable K 6.43 cmol(+) kg\(^{-1}\) soil\(^{-1}\), Na 1.09 cmol(+) kg\(^{-1}\) soil\(^{-1}\), Ca 2.70 cmol(+) kg\(^{-1}\) soil\(^{-1}\), and Mg 2.72 cmol(+) kg\(^{-1}\) soil\(^{-1}\), CEC 19.7 cmol(+) kg\(^{-1}\) soil\(^{-1}\), BS 69%, Mehlich 3 extractable P 6.87 g kg\(^{-1}\), K 8.91 g kg\(^{-1}\), Ca 14.5 g kg\(^{-1}\), Mg 3.97 g kg\(^{-1}\), Cu 6.22 mg kg\(^{-1}\), Pb 1.23 mg kg\(^{-1}\), and Zn 62.4 mg kg\(^{-1}\).

2.2. Incubation Experiment

To investigate the effect of biochar on soil chemical and fertility dynamics of compost excessive application soils, in this study, 5% (w/w) commercially available poultry-livestock manure compost was added as a soil fertilizer (twice the recommended amount of organic fertilizer in Taiwan). It should be noted that this is a highly unlikely scenario, given the economic non-viability of 5% (w/w) compost for most farmers, but that is not the objective of the present work.

The incubation experiment and the examination of inorganic nitrogen (NO\(_3\)−N and NH\(_4\)+-N) availability have been discussed in a previous study [25]. The effects of three proportions (0.5%,
1.0%, and 2.0% w/w) of biochar co-applied with compost (5.0% w/w) on SAO, MAI, and SAI soils were investigated over 371 days of incubation, in consistence with the study of C dynamic [24] but shorten incubation days. Control (0%) co-applied with compost (5.0% w/w) on SAO, MAI, and SAI soils were investigated. In total, twelve treatments were conducted in this study. For each treatment, biochar and compost were thoroughly mixed with stirring rods with the soils for at least 30 min. After mixing, a 25-g soil mixture was placed in plastic containers, each with a volume of 30 mL. The experiment had a completely randomized block design with 12 treatments, and each treatment had 110 replicates for destructive sampling during the incubation. The containers were sealed and incubated at 25 °C for 371 days, the soil moisture contents were adjusted to 60% of field capacity before the start of the incubation, and were maintained throughout the experiment using repeated weighing. The moisture was adjusted twice a week by weighing the jars and adding deionized water as necessary. The soil samples were destructively sampled from five replicate jars for each treatment; a series of 60 jars (3 soils × 4 amendments × 5 repetitions) was taken at days 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 77, 91, 105, 119, 133, 161, 189, 217, 245, 308, and 371 for analysis of available inorganic N (NO$^3$-N and NH$^4$+ N), total carbon, nitrogen (TN), phosphorus (TP), pH, electrical conductivity (EC), dissolved organic carbon (DOC), and available nutrients. Soil TC content was determined by dry combustion [28], using an OI Analytical Solid Total Organic Carbon (TOC) analyzer (OI Corporation/Xylem, Inc., College Station, TX, USA). Soil TC was assumed to be organic in nature because the low or neutral soil pH precludes carbonates. Soil TN content was extracted by digesting a 1.0-g dried and powdered sample using concentrated H$_2$SO$_4$ in a Kjeldahl flask using K$_2$SO$_4$, CuSO$_4$, and Se powder as catalysts. TN concentration was determined via OI Analytical Aurora Model 1030W analyzer (OI Corporation/Xylem, Inc., College Station, TX, USA); content of soil TP in the digested solution was determined with inductively coupled plasma optical emission spectrometry (ICP–OES) (PerkinElmer, Inc., Optima 2100DV, Waltham, MA, USA). The pH and EC were measured in 1:10 (w/v) soil to water slurry after sharked for 2 hr at 120 rpm. After pH and EC measurement, the slurry was centrifuged and filtered with 0.45-micrometer filter paper, and the filtered solution was used for DOC measurement by an Aurora 1030C TOC analyzer (OI Corporation/Xylem, Inc., TX, USA). Plant available nutrients, including P, K, Ca, Mg, Fe, Mn, Cu, Pb, and Zn, were determined by extracting 5 g (dry weight equivalent) of soil with 50 mL of Mehlich-3 extracting solution [29]. Mehlich-3 extractable (M3-) K, Na, Ca, Mg, Fe, Mn, Cu, Pb, Zn, and P values were measured with inductively coupled plasma optical emission spectrometry (ICP–OES) (PerkinElmer, Inc., Optima 2100DV, Waltham, MA, USA). Total inorganic nitrogen (TIN) was calculated as the sum of extractable NO$_3^-$-N and NH$_4^+$-N. The percentage of NO$_3^-$-N, NH$_4^+$-N, and nutrients that declined or increased due to biochar addition was calculated by the difference between biochar-amended treatments and un-amended control treatment [25,30].

2.3. Statistical Analysis

Statistical analyses (calculation of means and standard deviations, differences of means) were performed using the Statistical Analysis System (SAS) 9.4 package (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). A repeated measure multivariate analysis of variance (MANOVA) was used to test the changes in available nutrients with different biochar addition rates, soils, and incubation time. The addition rates and soils served as between-subject factors, and incubation time served as the within-subject factor. The repeated measure MANOVA was carried out using the general linear model (GLM) procedure. Results were analyzed by analysis of variance (MANOVA) to test the effects of each treatment. The statistical significance of the mean differences was determined using the least-significant-difference (LSD) tests based on a t-test at a 0.05-probability level. Values presented in graphs and text are means ± 1 standard deviation (SD). The Pearson correlation coefficient (r) was calculated, and principle component analysis (PCA) was performed using SAS 9.4 software. The multivariate statistical technique of PCA was used to investigate the most susceptible variances and to identify the important components explaining most of the variances in a large data set.
3. Results

3.1. Soil Properties and Available Nutrients

For the between-subject effects (Soil and Rate), the biochar treatments resulted in significant increase effect on pH, TC, TN, and C:N ratio and available K concentration, as well as a significant soil × rate interaction for C:N ratio in the soil (Table 1). Furthermore, for the within-subject effects during incubation time, the biochar treatments resulted in significant time, time × soil, time × rate, and time × soil × rate interactions for pH, DOC, TC, TN, TP, C:N ratio, and a significant time × rate interactions for available P and Fe concentrations in the soil.

From Figure 1, between biochar treatments and control, except for visible pH changes in SAI and visible EC changes in SAO, the effect of biochar on pH, EC, and DOC properties is overwhelmed by the effect of the excessive compost as time went on. Soil pH changes with incubation time was similar between biochar treatments and control in SAO and MAI, but it showed evident difference after day 250 with following order 2.0% > 1.0% > 0.5% > control. Also, soil EC changes with incubation time were similar between biochar treatments and control in MAI and SAI, but it showed evident difference after day 250 with following order control > 0.5% ≈ 1.0% ≈ 2.0%. During the incubation period, the pH in the SAO soil gradually reduced (Figure 1a). In the MAI and SAI soils, the soil pH showed a sharp decline at the beginning of incubation and high variability in the MAI soil. The decrease of the soil pH between days 1 and 371 was 2.2~2.4, 0.8~1.0, and 0.6~1.5 pH units for the SAO, MAI, and SAI soils, respectively. Increasing the amount of added biochar resulted in the reduction of changes in the soil pH, especially in the SAI soil. There was a minimal effect of biochar addition on the reduction of the soil pH changes in the MAI soil. The most significantly high EC value was observed in the MAI soil, followed by that in the SAO and SAI soils, but there was an insignificant difference between the SAO and SAI soils. The increase of the soil EC between days 1 and 371 was 0.2 dS m$^{-1}$ for the SAO soil and 0.1~0.2 dS m$^{-1}$ for the MAI and SAI soils (Figure 1b). The DOC content gradually declined with an increase in incubation time (Figure 1c). The decrease of the DOC between days 1 and 371 was 239~272, 82~105, and 127~148 mg kg$^{-1}$ for the SAO, MAI, and SAI soils, respectively.

From Figure 2, between biochar treatments and control, except for significant TC and C:N ratio changes in all soils with the following order 2.0% > 1.0% > 0.5% > control, the effect of biochar on TN and TP is also overwhelmed by the effect of the excessive compost as time goes on. The decrease of the TC between days 1 and 371 was 0.3~0.8, 0~0.1, and 0.2~0.6% for the SAO, MAI, and SAI soils, respectively (Figure 2a). The difference in TN content between days 20 and 30 was about 0.5g kg$^{-1}$ (Figure 2b). A previous study [25] indicated that higher nitrate content was measured between days 20–30; that soil sample collection time was extended (7 days) could be one of the influence factors. We speculated that a slight increase of the microbial biomass could occur during days 20-30, resulting in temporary fixation of nitrate and a slight increase in TN content. The difference of TN and TP between days 1 and 371 was 1.0~1.4 and 1.0~1.4, 0.4~1.0 and 0.3~0.6, and 0.9~1.0, and 0.4~1.2 g kg$^{-1}$ for the SAO, MAI, and SAI soils, respectively (Figure 2b,c). Increasing the biochar addition resulted in a significant increase in the C:N ratio in the three soils. During the incubation period, the C:N ratio slowly declined in the three soils (Figure 2d). The decrease of the C:N ratio between days 1 and 371 was 4.0~6.0, 0.7~4.3, and 4.4~7.1 for the SAO, MAI, and SAI soils, respectively.
Table 1. Significance (p-value) of repeated-measures MANOVA results on soil properties and Mehlich 3 extractable nutrients concentrations in different soil series (Soil) and biochar application rates (Rate) in this study.

| Source of Variation | df | pH  | EC  | DOC | TC  | TN  | TP  | C:N | P   | K   | Ca  | Mg  | Fe  | Mn  | Cu  | Pb  | Zn  |
|---------------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Between subject effect |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Soil                | 2  | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   |
| Rate                | 3  | *   | ns  | *   | *   | ns  | *   | ns  | *   | ns  | ns  | ns  | ns  | ns  | ns  | ns  |
| Soil × Rate         | 6  | ns  | ns  | ns  | ns  | ns  | ns  | ns  | *   | ns  | ns  | ns  | ns  | ns  | ns  | ns  |
| Error               | 48 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Within-subject effect |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Time                | 21 | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   |
| Time × Soil         | 42 | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   | *   |
| Time × Rate         | 63 | *   | ns  | *   | *   | *   | *   | *   | ns  | ns  | ns  | *   | ns  | ns  | ns  | ns  |
| Time × Soil × Rate  | 126| *   | ns  | *   | *   | *   | *   | *   | ns  | ns  | ns  | ns  | ns  | ns  | ns  | ns  |
| Error               | 1008|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

Notes: EC = electrical conductivity; DOC = dissolved organic carbon; TC = total carbon; TN = total nitrogen; TP = total phosphorus; df = degree of freedom. The asterisks (*) indicate the significant difference at \( p < 0.0001 \); ns = not significant.
Figure 1. Effects of biochar additions on soil properties: (a) pH, (b) electrical conductivity (EC), and (c) dissolved organic carbon (DOC) during a 371-day period. The data are mean value ($n = 5$), and vertical bars represent standard deviations (SDs) of the means.

Figure 1. Effects of biochar additions on soil properties: (a) pH, (b) electrical conductivity (EC), and (c) dissolved organic carbon (DOC) during a 371-day period. The data are mean value ($n = 5$), and vertical bars represent standard deviations (SDs) of the means.
Figure 2. Effects of biochar additions on soil properties: (a) total carbon (TC), (b) total nitrogen (TN), (c) total phosphorus (TP), and (d) C:N ratio during a 371-day period. The data are mean value ($n = 5$), and vertical bars represent standard deviations (SDs) of the means.
3.2. Principal Components (PCs) and Correlation Coefficients

In this study, PCA was performed to investigate the important components in the large data set and the different parameters, including NO$_3$$^-$-N, TIN, soil pH, EC, DOC, TC, TN, C:N ratio, Mehlich 3-extractable P and K at the beginning (day 3) and end (day 371) of the incubation, were introduced as the analysis variables in the PCA. The results are shown in Table 2. From the PCA, the PC1 and PC2 explained 34.5% and 21.3%, 30.2% and 23.4%, and 38.2% and 22.6% of the total variance in SAO, MAI, and SAI soils at day 3, accounting for 55.8%, 53.6% and 60.8% of the total variance, respectively. At day 371, the PC1 and PC2 explained and accounted for 68.5%, 66.9%, and 61.9% of the total variance in SAO, MAI, and SAI soils, respectively. In SAO soil, the PC1 had significantly positive correlations with the contents of NO$_3$$^-$-N and TIN at day 3, and P and K content were significantly positively correlated with PC2. In MAI soil, the PC1 had significantly positive correlations with the contents of NO$_3$$^-$-N, TIN, and TN, and no parameters were significantly positively correlated with PC2. In SAI soil, at the beginning of incubation, TC and C:N ratio were found to be significantly positive correlations with PC1, but P content showed a significantly negative correlation. NO$_3$$^-$-N and TIN content had significantly positive correlations with PC2.

| Parameter      | SAO Soil     | MAI Soil     | SAI Soil     |
|----------------|--------------|--------------|--------------|
|                | Day 3 | Day 371 | Day 3 | Day 371 | Day 3 | Day 371 |
| PC1            |        |        |        |        |        |        |
| NO$_3$$^-$-N   | 0.78   | -0.11  | -0.51  | -0.18  | 0.80  | -0.47  | -0.93  | -0.07  | -0.11  | 0.94  | -0.77  | 0.41   |
| TIN            | 0.78   | 0.38   | -0.79  | -0.19  | 0.83  | -0.41  | -0.93  | -0.09  | -0.11  | 0.91  | -0.78  | 0.38   |
| pH             | 0.68   | -0.02  | 0.82   | 0.10   | 0.65  | 0.57   | 0.85   | 0.03   | 0.57   | 0.20  | 0.93   | 0.01   |
| EC             | 0.57   | -0.31  | -0.80  | 0.11   | -0.28 | -0.22  | 0.26   | -0.06  | 0.58   | 0.46  | -0.13  | 0.27   |
| DOC            | 0.30   | 0.20   | 0.59   | 0.27   | 0.15  | -0.28  | -0.34  | 0.54   | 0.35   | -0.37 | 0.05   | 0.79   |
| TC             | -0.22  | -0.01  | 0.83   | -0.22  | 0.27  | 0.75   | 0.95   | -0.09  | 0.93   | 0.13  | 0.92   | 0.26   |
| TN             | 0.34   | -0.21  | 0.84   | -0.24  | 0.83  | 0.28   | 0.44   | -0.51  | 0.52   | -0.30 | 0.36   | 0.66   |
| C:N            | -0.24  | 0.14   | 0.73   | -0.20  | -0.27 | 0.54   | 0.92   | -0.02  | 0.92   | 0.16  | 0.94   | 0.13   |
| P              | 0.05   | 0.95   | -0.06  | 0.95   | -0.59 | -0.01  | -0.01  | 0.88   | -0.80  | 0.12  | 0.02   | 0.46   |
| K              | 0.06   | 0.93   | 0.16   | 0.85   | 0.04  | 0.75   | 0.53   | 0.68   | -0.61  | 0.10  | -0.01  | 0.70   |

Notes: EC = electrical conductivity; DOC = dissolved organic carbon; TC = total carbon; TN = total nitrogen. The asterisks after the data indicate the significant correlations analyzed by SAS (*p < 0.0001).

4. Discussion

4.1. Effect on Soil pH, EC, and DOC

The increasing biochar application rate caused a slight increase in soil pH and DOC and a slight decrease in soil EC (Figure 1). The increase in soil pH depended on the biochar and the buffering capacity of the soil, which is its ability to resist changes in pH and depends on several factors, including the soil’s organic and mineral content and its physical properties [31]. Therefore, the soil pH has been shown to change in response to the addition of biochar [4,31–35]. Change in pH over time, averaged over biochar rate, decreased soil pH by a maximum of 2.3, 0.86, and 0.90 pH units for SAO, MAI, and SAI soil, respectively (Figure 1a). The initial soil, biochar, and compost pH values were 6.1 (SAO), 7.5 (MAI), 6.5 (SAI), 9.9, and 8.4, respectively. Biochar may increase soil pH due to its inherent alkalinity, and thus it has become a promising amendment for acidic soils [36]. In addition, soil buffering capacity prevented major changes in soil pH, even at the highest biochar application rate (10%, w/w) [37]. According to the previous study results [24,27], the C half-life of control, 0.5%, 1.0%, and 2.0% in SAO, MAI, and SAI soils was 44, 42, 44, and 43, 58, 60, 55, and 61, and 57, 57, 55, and 59 days, respectively. Biochar co-application has no evident effect on the reduction of decomposition of excessive compost. That is, in the current study, at the beginning of incubation, excessive compost addition has obviously influenced and increased soil pH, followed by biochar addition. During the incubation, excessive
compost was decomposed quickly during 50–60 days in studied soils, and the ability to increase soil pH declined. The SAO soil is a red earth with lower pH. The excessive compost decomposed would release more acidic materials and result in more acidic SAO. The MAI soil and SAI soil are both fluvio-aquic soil but with different pH. The mildly alkaline MAI has a higher buffering capacity by calcium ion (Ca$^{2+}$), the decline with time was less evident. The acidic SAI soil showed similar changes to MAI soil before day 200. After Day 200, much more decomposed compost continually made control acidification, but soil pH of biochar treatments was evidently higher than control, indicating the influence of the inherent alkalinity of biochar could sustain with time. It is clear that excessive compost addition can strongly affect soil pH at the beginning of the incubation but quickly decomposed with time. The buffering capacity in MAI soil and the inherent alkalinity of biochar could have the ability to prevent soil pH changes, even at the 0.5% biochar addition.

The temporal change of EC values during incubation was obvious in SAO soil, followed by SAI and MAI soil. Such EC increases could be attributed to the release of basic cations from excessive composts. A similar result was reported by Chintala et al. [38] and Berek et al. [12]. Berek et al. [12] indicated that the combined additions of biochar (2%) and compost (2%) significantly increased pH and EC. Lentz and Ippolito [39] showed no difference in EC between a control and a 22.4-Mg biochar ha$^{-1}$ under field conditions. Increasing the biochar application rate caused a decrease in soil EC [37], and the results could be attributed to salt sorption by biochar [40,41]. Our study results indicated that salt sorption occurred evidently in SAO soil, and could be attributed to the higher clay content. In addition, the specific surface area (BET) of the studied biochar was low (36.6 m$^2$ g$^{-1}$) [26] and lacked a surface functional group [27], both indicating the low sorption capacity. We examined that the studied biochar cannot be expected to have significant salt sorption ability.

The amount of DOC showed a slight increase as the rate of biochar application increases, and the effect of biochar on DOC was similar to soil pH. The addition of 0.5% biochar induced a raise in DOC of up to 6% in MAI soil. In the case of the biochar addition rate of 1.0% and 2.0%, DOC raised by up to 7.2% in MAI soil and 12% in SAI soil. The previous study results indicated that biochar treatments significantly reduced C mineralization in SAO soil, and showed an insignificant difference in MAI soil, but significantly increased C mineralization in SAI soil (1.0% and 2.0% treatments) [24]. In contrast, the decline in the amount of DOC as the rate of biochar application increases has been reported [30,42], especially in soil with relatively high soil organic matter and/or inorganic nitrogen and/or DOC. In our study, the decreasing effect of biochar addition on soil DOC is not significant because of the very low CEC (about 9, 12, and 14 cmol(+)$^{-1}$ kg$^{-1}$ for SAO, MAI, and SAI soil), which suggests low soil organic matter in the three studied soils. Furthermore, the DOC of control and biochar treatments continued to decline in this study (Figure 1c). This finding agrees with Zimmerman et al. [43] and Hailegnaw et al. [30]. In the current study, soils with excessive compost application could result in more decomposition of organic matter (compost) and/or the sorption of DOC resulting from the decomposition. In addition, Shen [44] suggested that the sorption capacity of dissolved organic matter (DOM) for soils appears to be related to the clay content of the soil, with a high soil clay content favoring the sorption process, and DOM sorption was found to be maximum at pH 4-5 and to decrease with a further increase of pH. The lower pH and high clay content of SAO soil seemed to absorb more DOC resulting from the decomposition of excessive compost, followed by SAI and MAI soil. The higher DOM sorption may also be due to the higher content of extractable Al and Fe in soil [31], and the sorption of hydrophobic DOC by oxide/hydroxide of soils possibly due to the higher affinity of hydrophobic DOC to oxide/hydroxide of Al and Fe [45]. The content of dithionite-citrate-bicarbonate extractable Al and Fe was 5.95 and 25.7, 1.23 and 10.5, and 0.93 and 6.96 g kg$^{-1}$ for SAO, MAI, and SAI soil, respectively (unpublished data), which showed the potential for absorbing DOC. In addition, at the beginning of the incubation, the increase in soil pH of control and biochar treatments can result in the increased solubility of organic matter and growth of the negative charge of organic matter. Whittinghill and Hobbie [46] suggested that the increment in solubility and/or negative charge on the organic matter results in the stabilization of negatively charged organic matter by sorption to
positively charged cations. The formation of a Ca\textsuperscript{2+} bridge between negatively charged particles may bind organic matter together or to minerals [44]. We think this phenomenon may have occurred in our incubated soils with the addition of compost and biochar very rich in exchangeable Ca\textsuperscript{2+} ions (Table S1) and released to the soil solution, possibly forming Ca\textsuperscript{2+} bridging with soil organic matter (SOM).

### 4.2. Effect on Soil TC, TN, TP and C:N Ratio

As expected, the application of biochar in excessive compost-fertilized soil significantly increased soil TC concentration in comparison with the application of excessive compost alone, probably because biochar is made up mostly of C (Table S1). Rapid turnover of organic matter results in low efficiency of organic fertilizers that are used to increase and sequester C in soils in humid tropical climates [1]; the key benefits of using biochar in soil is that C offset credits can be easily and accurately quantified based on the amount of biochar C that is applied and that biochar C is very stable [13]. Our results are consistent with the previous research results [24,39,47–49].

In contrast, the application of biochar in excessive compost-fertilized soil has no evident increase in soil TN in comparison to the control. Masunga et al. [50] indicated that in most cases, soil is N-deficient because it contains little or poor-quality organic matter, and the addition of organic amendments, which are usually N-rich, to soil usually improves the quality of soil organic matter. The co-application of biochar and paper mill biosolids did not increase total soil N in comparison with the application of paper mill biosolids alone, probably because of the low N content of the biochar [49]. In this study, the N content of biochar and study soils was low (Table S1). The studied compost is a high-quality organic matter with a low C:N ratio and with enough N, and its application into soil can result in releasing available N via N mineralization. The research results of N availability [25] indicated that increased biochar addition resulted in an obvious decrease in total inorganic nitrogen content (available nitrate and ammonium), but insignificantly. The reports of Luo et al. [36] showed that the soil TN content increased by 17.1% and 80.9% in the 1%BC and 3%BC treatments compared with the control, respectively. Soil TP content showed similar results with soil TN content, but the increase was higher than TN. In contrast, Li et al. [47] indicated that the soil TP content depended on the soil N level and the rate of biochar addition as well as their interaction \((p < 0.05)\).

The changes of C:N ratio were similar to soil TC, which showed a significant increase with increased biochar rate. Robertson and Groffman [51] indicated that the C:N ratio, reflecting the content of available C and N for organisms, is a key factor controlling the balance between N mineralization and N immobilization, occurring at the same time within relatively small volumes of soil. Joseph et al. [52] pointed out the mineralization of organic nitrogen after field application depends on a number of factors, including the type and organic composition of organic residues (C, N, C:N ratio), soil type and properties, soil temperature and water content, and environmental conditions; the amount of nitrogen available to crops is also influenced by the application rate, and by the timing and method of application. In addition, the mineralization and release of biochar will be dependent on how recalcitrant the biochar and soil N and C pools are, and on the soil and biochar C:N ratio [53]. The feedstock, C:N ratio of biochar, C:N ratio of the co-amended organic substrate, and the abundance of substrates that can be used by soil microorganisms are several factors affecting N immobilization [54]. Woody biochar is assumed to stimulate N immobilization, but because woody biochar is more recalcitrant than other biochars, the extent of stimulation remains uncertain [55,56]. The C:N ratio of studied biochar was 97:1, much greater than the assumed approximately 20:1 [57], 25:1 [51], or 35:1 [58] minimum ratio when immobilization response is observed, implying that the additions of the biochar may increase the C:N ratio, thus causing N immobilization. In the biochar treatments, the C:N ratio significantly increased during the incubation (Figure 2d), but it was lower than 14 in three soils. The reduction of N mineralization was inconsistent with our results [25], and could be attributed to the low C:N ratio of blended biochar, compost, and soil mixture, implying that N mineralization happens. The literature reports have suggested that the immobilization of N following the addition of pinewood biochar with a high C:N ratio [59], the immobilization of mineral N of up to 43% following biochar application [60],
and the amount of decline increased as the rate of biochar application increased from 0.5% to 8% [30]. In this study, the C:N ratio of studied biochar was high (97:1); however, when biochar was mixed with excessive compost and soil, the very minimal extent sorption that occurred could not explain the significant decrease of N mineralization. Similar results have been reported by Dempster et al. [61] and suggested that this is attributed to the small proportion of biochar within the soil matrix. Furthermore, except for the relatively low application rate, low specific surface area and lack of surface functional group could also be the two main impact factors.

4.3. Effect on Soil-Available Nutrients

Incorporation of biochar and compost into acid soils would increase nutrients content in the soils [12]. Mehlich-3 extractable P, K, Ca, Mg, Fe, and Mn in the Ultisols increased by 1478%, 2257%, 1457%, 258%, 308%, −14%, and −36%, respectively. The increase of soil nutrients is attributed to [12] (1) the release of such nutrients directly from the compost or biochar (as nutrient sources); nutrients such as N, P and K were released by composts and some nutrients (especially K) were released by biochars; (2) changes in soil properties by biochar, e.g., the increase of soil pH that can solubilize nutrients such as P or precipitate Al and Fe; (3) adsorption of nutrients by surface charge or micropore of biochar and the complexation of Al and Fe by organic acids and functional groups of composts and/or biochars; (4) decreasing of nutrient leaching such as NO$_3^-$ and P by improving water-holding capacity of the amended soils, and (5) the formation of organic coating in the outer and inner pores of biochar when it is co-composted or added in combination with compost, which could act as a glue for water and nutrient retention.

The amount of available P increased as the rate of biochar application increased from 0.5% to 2% in SAO soil. The obvious increment of P content in MAI soil and SAI soil was the addition of 1.0% BC (on average 1.7%) and 0.5% BC (on average 3.5%), respectively. Li et al. [47] also indicated that the application of biochar had a greater effect on available P, resulting from increased application rates. The P retention was a function of surface functional groups, the presence of Fe and Al oxides, and precipitation with Ca and Mg [7]. The decrease in available P concentration upon biochar application may be caused by P sorption to biochar surfaces [62]. However, low specific surface area and lack of surface functional group both suggested that the amount of P adsorbed on studied woody biochar would lower than that on soil surfaces. Zhai et al. [63] reported that application of 2–8% biochar significantly increased both Olsen-P and soil microbial biomass (SMB)-P in red earth and fluvo-aquic soil, with greater effect by increasing application rates, and the different increase in Olsen-P values in two soils after amendment with the same rate of biochar depends on differences in the chemical properties of the two soils. The P sorption in red earth, being an acidic soil, is controlled by the contents of Fe and Al, whereas in the alkaline fluvo-aquic soil, phosphate can precipitate as Ca and Mg phosphates. The SAO soil is a red earth, a common rural soil in Taiwan, with a strong P fixation that largely constrains the supply of P to crops, and improvement of soil P availability is of especial importance for this type of soil. In the current study, the increase in soil P availability after biochar application is greater in SAO soil with lower P sorption capacity. Furthermore, the results by Takaya et al. [64] indicated some positive correlations between available P adsorption and the Ca or Mg contents of biochar, and the other studies have suggested that the presence of surface MgO and other cations, including Ca$^{2+}$ and Al$^{3+}$, is also known to improve phosphate adsorption [65,66]. The MAI soil and SAI soil both are fluvo-aquic soil. The MAI soil, being slightly alkaline, has the highest content of available P, K, and Ca (Table S1), and the SAI soil, being slightly acidic, has the highest content of available Mg and Fe. We speculated that the phosphate in MAI soil and SAI soil can precipitate as Ca, and Mg and Fe phosphates, respectively.

The woody biochar added K, Ca, and Mg to the soil (Table S1). The percentage of changed K, following biochar addition in three soils relative to the control, indicated that the significant increase effect of biochar was prominent during the incubation period. The addition of biochar resulted in the
increment of K by up to on averaged 0.4%~1.0% for 0.5% BC, 0.7%~5.1% for 1.0% BC and 6.2%~7.6% for 2.0% BC, respectively, relative to the control. Furthermore, although the application of woody biochar added Ca and Mg to the soils, it slightly increased the amounts of available Ca in three soils and Mg in SAO and MAI soil, but slightly reduced the Mg concentrations in SAI soil. The results of the effect of peanut hull and pine chip biochars on soil nutrients indicated that the peanut hull biochar linearly increased Mehlich I K, Ca, and Mg in the surface soil (0~15 cm), and the pine chip biochar had no effect on other nutrients except Mehlich I Ca [3]. Nelson et al. [62] indicated that the Ca and Mg present in the woody biochar probably were not available for plant uptake.

The woody biochar did not add numerous Fe, Mn, Cu, Pb, and Zn to the soil, and the effect of the woody biochar on Fe, Mn, Cu, Pb and Zn availability in three soils was insignificant. Laird et al. [6] found that biochar treatment had no significant effect on Mehlich 3 extractable Cu and Zn concentrations, but Mn, Cu and Zn availability (by Mehlich 1 extraction method) was decreased by 17% and increased by 33% and 23%, respectively, when 2% biochar was mixed into the soil [67]. The decrease in trace element concentrations in the biochar-amended soil may have been associated with cation adsorption on biochar surfaces [67]. The effect of biochar on trace element uptake and availability can vary with the trace element species and biochar application rate, such as increasing extractable As and Zn concentrations, not affecting extractable Cu concentration, reducing extractable Pb concentration, and having inconsistent effects on extractable Cd concentration in soil [68]. The increase in extractable soil Zn at the higher (2% and 10%) as compared to the lower (0% and 1%) biochar application rates was a synergistic effect between biochar and manure [11]. Ippolito et al. [37] suggested that in alkaline soils, Zn and Cu form strong associations with Fe and Al (hydr)oxides and thus may limit their availability. Over time, in the current study, available Fe, Mn, Cu, and Zn concentrations showed variable changes (data not shown), and could be attributed to Fe and Mn mineral forms changing and soil pH changing, as suggested by Ippolito et al. [11,37]. In SAI soil, the soil pH increased and maintained at above pH 7.0 until day 119, which can explain the metal extractability decrease. Furthermore, the low BET and less surface functional group of the studied biochar would cause insignificant adsorption of Fe, Mn, Cu, Pb, and Zn.

5. Conclusions

In the current study, we examined the effects of biochar-excessive co-application on soil nutrient status and evaluated the regulation or enhancement role of biochar application rates in three rural soils over time. Our study showed that for between-subject effects (Soil and Rate), the biochar treatments resulted in significant rate for pH, TC, TN, C:N ratio, and available K concentration, and in a significant soil × rate interaction for the C:N ratio in the soil. As to the within-subject effect during incubation time, the biochar treatments resulted in significant time, time × soil, time × rate, and time × soil × rate interactions for pH, DOC, TC, TN, TP, C:N ratio, and significant time × rate for available P and Fe concentrations in the soil. The increasing biochar application rate, averaged over time, caused an insignificant change but a slight increase in soil pH and DOC, a slight decrease in soil EC, a significant increase in soil TC content, and an insignificant change in soil TN and TP content. The N immobilization was less prevalent, most likely due to the excessive compost application. The amount of available P showed a slight increase as the rate of biochar application increased from 0.5% to 2% in SAO. The P sorption in SAO, being an acidic soil, is controlled by the contents of Fe and Al, whereas in the SAI and MAI, phosphate can precipitate as Ca and Mg phosphates. The addition of biochar resulted in the increment of K by up to on averaged 0.4%~7.6% for 0.5%~2.0% BC, relative to the control. In addition, the woody biochar did not add numerous Ca, Mg, Fe, Mn, Cu, Pb, and Zn to the soil, and the effect was insignificant. The concentration of Cu, Pb, and Zn showed a slight increase in SAO and MAI but an evident decrease in SAI, especially for Pb. Based on the results, we rejected our hypothesis that biochar co-application could have positive effects on the absorption of excessive nutrients Cu and Zn in excessive compost-fertilized soil. The impact of excessive compost (5% by wt) on soil was much higher than the relatively low biochar application rates (<2% by wt), and thus eliminated the effect of
biochar. In addition, the low specific and less surface function group of studied biochar could be the two important factors. In the condition of no effects on soil nutrient status, biochar co-application is still advantageous in carbon sequestration and N conservation in excessive compost-fertilized soils in Taiwan.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/5/683/s1. Table S1: Characteristics of biochar, compost, and the three studied soils.

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