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Nutrient Dynamics in Sandy Soil and Leaf Lettuce Following the Application of Urea and Urea-Hydrogen Peroxide Impregnated Co-Pyrolyzed Animal Manure and Bone Meal

Deogratius Luyima [1], Michael Egyir [1], Yeo-Uk Yun [2], Seong-Jin Park [3,*] and Taek-Keun Oh [1,*]

[1] Department of Bio-Environmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 34134, Korea; deoluyima@gmail.com (D.L.); miki.egyir@gmail.com (M.E.)

[2] Division of Environmentally Friendly Agriculture, Chungcheongnam-Do Agricultural Research and Extension Services, Yesan-gun 32418, Korea; aoggi61@korea.kr

[3] Division of Soil and Fertilizer, National Institute of Agricultural Science, RDA, Wanju 55365, Korea

* Correspondence: archha98@korea.kr (S.-J.P.); ok5382@cnu.ac.kr (T.-K.O.)

Abstract: There is a paucity of data regarding the effect of nutrient-enriched biochar amendments on nutrient dynamics in both soil and crops. This is important because unlike pristine biochar, nutrient-enriched biochar is applied to the soil in minute quantities as large amounts may led to over application of the nutrients loaded in it. The current study examined the effects of both phosphorus- and nitrogen-enriched biochars on the dynamics of both macro and micronutrients in the sandy soil and leaf lettuce grown thereon. The phosphorus enrichment followed co-pyrolysis of animal manure (cow dung) with 25% and 50% bone meal (w/w), while the nitrogen enrichment was achieved by soaking the co-pyrolyzed biochar into urea and urea-hydrogen peroxide. The performances of the nutrient-enriched biochar were compared with the conventional amendment of urea and triple superphosphate (TSP) in the production of leaf lettuce over a period of two seasons in a pot experiment. The nutrient-enriched biochar amendments resulted into higher microbial biomass carbon and carbon to nitrogen ratios than the conventional amendment. The conventional amendment caused more phosphorus, potassium, and magnesium accumulations in the leaf lettuce than the nutrient-enriched biochar amendments. The nutrient-enriched biochar amendments led to more accumulations of nitrogen, calcium, and micronutrient elements in the leaf lettuce and availabilities of all the nutrient elements in the soil and thus, nutrient-enriched biochar acted as a reservoir that could provide nutrients to the growing lettuce beyond a single growing season.

Keywords: leaf lettuce; nutrient availability; nutrient dynamics; nutrient-enriched biochar

1. Introduction

Sandy soils characterized by sand contents of more than 68% and clay contents of less than 18% are strongly acidic in nature [1]. Chronic deficiencies of nutrients, especially nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and micronutrients of molybdenum (Mo), zinc (Zn), and boron (B), compound into infertility of these soils and low crop productivity [2]. The use efficiencies of the fertilizers applied to such soils are extremely low as the different nutrients are lost through an assortment of pathways. For example, cationic nutrient elements are lost mainly through leaching and phosphorus is strongly fixed, while surface runoff accounts for a considerable portion of the loss of all nutrients [1]. To avert the environmental perils and economic loss ensuing from the low use efficiencies of the fertilizers by crops grown on such soils, the idea of formulating slow release fertilizers has been mooted and several research studies have been conducted to discern the efficacies of the slow release fertilizers prepared in different ways.

Despite the enormous benefits that can accrue from the usage of such fertilizers, their utilization is still tremendously low, accounting for just less than 1% of the total quantity of

fertilizers consumed globally every year [3]. This low level of utilization mainly stems from the fact that they are expensive and their production involves sophisticated technology [4]. Lammel et al. [5] for example, indicated that most common polymer-coated fertilizers are about 8–12 fold more expensive than the conventional mineral fertilizers. Additionally, some of the coating materials used in the formulation of the slow release fertilizers are non-biodegradable, while others are mutagenic and/or carcinogenic [4,6,7]. Because of the issues mentioned above, a lot of research effort has been geared towards exploring environmentally friendly and cost-effective carriers and coating materials for the plant nutrients [4].

There have been proposals to use amply available low-cost lignocellulosic crop residues such as wheat straw with high contents of lignin, hemicellulose, and cellulose [8] for the preparation of slow release fertilizers, by, for example, Xie et al. [9] and Liu et al. [10]. However, lignocellulose and compost are subject to rapid decomposition once incorporated into soil [11]. For that matter, carbon-based materials such as, biochar and lignite, have lately gained copious interest with several studies conducted to assess their potential as coating agents and fertilizer nutrient carriers [4]. The growing interest in using carbon-based materials to prepare the slow release fertilizers is premised on their biodegradability and additional benefits they render when applied to the soil, including improving soil physical, chemical, and biological properties as well as sequestering carbon from the atmosphere [4]. Of all the carbon-based materials, biochar has attracted the most interest for usage in the formulation of slow release fertilizers and several biochar-based slow release fertilizers have been formulated and studied for their agronomic efficiencies. This huge interest arises from the fact that the pyrolysis process turns carbon captured by plants during photosynthesis into recalcitrant forms with prolonged residence times of up to >1000 years in the soil.

However, animal manure feedstock biomasses have very low carbon retention capacities when charred [12] and co-pyrolysis with P has been mulled as an effective way of increasing the carbon content of the animal manure biochar by Zhao et al. [13] and others. Since phosphate rock reserves are dwindling [14], searching for renewable sources of phosphorus for future use is imperative. It is against this background that [12] proposed the usage of bone waste and discerned that the biochar obtained from the co-pyrolysis of cow dung and bone meal retained much more carbon than the pristine cow dung biochar. Apart from increasing the amount of carbon (C) retained in the biochar, co-pyrolysis of biomass with P also slows down the rate of discharge of P and it is thus an effective way of formulating a slow release P fertilizer as confirmed by Zhao et al. [13] and Luyima et al. [7]. It is worthy to note, however, that adsorption is a crucial mechanism in the preparation of slow release fertilizers [4], which may explain why the vast majority of the biochar-based fertilizers have been formulated with salts containing charged elements such as ammonium, nitrate, phosphates, K, etc. However, since urea is the most widely used nitrogen fertilizer in the world [15,16], its efficient management (checking its inordinate release into the environment) is of utmost importance for both agricultural productivity and environmental quality [17].

It is against this backdrop that our previous studies, i.e., Luyima et al. [7,12] proposed an easy way of formulating slow release of nitrogen and P through co-pyrolysis of cow dung (a representative of animal manure) with bone meal and sorption of the biochar in urea and urea-hydrogen peroxide solutions. The formulated urea-enriched biochars were efficient at reducing ammonia emissions as well as lessening N and P releases and boosting agronomic efficiency. Additionally, these fertilizers can be a rich source of all the nutrients lacking in the acidic sandy soils, since both bone waste and animal manure are laden with macro-, meso-, and micro-nutrients [18,19]. Although it is general knowledge that biochar influences the nutrient status of the soil and crops as confirmed by several studies, for example by Güereña et al. [20], Biederman and Harpole [21], Karimi et al. [22], and others, there is hardly any data concerning the nutrient statuses of both the sandy soils and crops following application of nutrient-enriched biochars. This is crucial because

nutrient-enriched biochars are applied in minute quantities to the soil and may, therefore, not elicit the same changes in the soil and crops as pure biochars. The present study was conducted to delineate whether or not the nutrient-enriched biochars improved the availability and uptake by leaf lettuce of the nutrients lacking in the acidic sandy soils.

2. Materials and Methods

2.1. Nutrient Dynamics Parameters Assessed

To assess P dynamics, the amount of P accumulated into the leaf lettuce, root to shoot ratios, and the ratios of Olsen P to total P in the soil were determined. The parameters used for the determination of N dynamics assessed included; the amount of N accumulated into the leaf lettuce, nitrate N content of the soil after lettuce harvests, and the percentage contribution of the first season's remnant N to the total quantity of N accumulated into the leaf lettuce in the second season. Additionally, the quantities of other major nutrient elements including K, Ca, and Mg as well as selected micronutrient elements including B, Mo, and Zn accumulated into the leaf lettuce, and the quantities remaining in the soil after each lettuce harvest were also assessed. Last but not least, the changes in the soil microbial biomass C, N, and C:N ratios elicited by the applications of the NEBs fertilizers and other amendments were also assessed.

2.2. Preparation of the Enriched Biochars and Experimental Set-Up

The urea-enriched biochars were produced as outlined by Luyima et al. [7]. Briefly, cow dung was dried and strained through a 2 mm sieve, after which bone meal was added and mixed thoroughly. Bone meal was added at two different concentrations of 25 and 50%. The mixture containing 25% bone meal was denoted as BM 25, while a mixture that contained 50% bone meal was symbolized as BM 50. Both mixtures were pyrolyzed at 500 °C, cooled, and each soaked in both urea and urea-hydrogen peroxide (UHP) solutions. The mixtures were then oven-dried at 105 °C for 24 h. The BM 25 mixtures soaked in urea and UHP solutions were denoted as BM 25+Urea and BM 25+UHP, respectively. On the other hand, BM 50+Urea and BM 50+UHP denote BM 50 mixtures that were soaked in urea and UHP solutions, respectively. The nutrient-enriched biochars (NEBs) were then used to grow leaf lettuce (Romaine variety) in a pot experiment that was carried out in a glasshouse at Chungnam national university, Daejeon, South Korea. The leaf lettuce was grown for two seasons. Each pot contained 3.5 kg of soil. The soil used belongs to the Inceptisol and Udepts order and suborder, respectively, according to the IUSS working group WRB classification. The NEBs were applied to the soil at a rate of 0.5% (w/w) in both seasons, while the mineral fertilizers were applied at rates recommended by the Ministry of Agriculture of South Korea. The bone char (BC) was added at rates that fulfilled the phosphorus requirements of the leaf lettuce as stipulated by the Ministry of Agriculture of South Korea. The performances of the NEBs were compared with those of the conventional mineral fertilizer amendment of urea and triple superphosphate (Urea+TSP), while a treatment with bone char (BC) as a source of phosphorus (Urea+BC) was also included. Each treatment was set-up in two different sets with each set replicated thrice. One of the sets was used as a control in the second lettuce-growing season and thus, each treatment had its own control experiment in the second season. Each lettuce-growing season lasted for five weeks after transplanting two-week old seedlings, which had been raised on amendments similar to those of their respective pots. The seeds of the leaf lettuce were purchased from Aramseed, Seoul, South Korea. All the amendments were classified into four P sources including; BM 25, BM 50, BC, and TSP. BM 25 values were computed as averages of both the BM 25+Urea and BM 25+UHP amendments with BM 50 computed in the same way.

2.3. Labotory Methods Used for the Assessment of NEBs, Soil and Plant Tissues

The surface properties and functional groups of the BM biochars and their corresponding NEBs were determined by both the Fourier transform infrared (FT-IR) spectrometer

(Nicolet 6700, Thermo Scientific, Waltham, MA, USA) and the scanning electron microscope and the results are presented in Figures S1 and S2 in the Supplementary File. P, Ca, Mg, K, and Zn were extracted from the dry ashed leaf lettuce samples using HCl as outlined by Kalra [23]. Briefly, 1.0 g of the air-dried leaf lettuce was weighed into a porcelain crucible and ashed in a muffle furnace at 500 °C for 4 h. The ashes were cooled and dissolved in 20.0 mL of 1.0 M HCl and then analyzed for the above-mentioned elements in the ICP-OES after dilution of the solution to 100 mL with distilled water. Both B and Mo were determined colorimetrically, where by Mo was determined following a method espoused by Kalra [23], whereas B was determined using the Azomethine-H method outlined by Wolf [24]. Ca, Mg, and K contents of the soil were extracted with 1.0 N neutral ammonium acetate solution and determined with ICP-OES as outlined by Jones [25]. Zn contents of the soil were extracted with Mehlich No. 3 solution as espoused by Jones [25] and determined with ICP-OES. Soil-available Mo was determined colorimetrically following a method developed by Fontes et al. [26], while soil-available B was determined by the Wolf [24] method. The Olsen P content was determined colorimetrically with a UV-Vis spectrophotometer at 880 nm following the ascorbic acid method espoused by Murphy and Rilley [27] after extraction with the Olsen reagent. The total soil P was determined following the same above-mentioned method, but the extraction was done by perchloric acid digestion as outlined by Gasparatos and Haidouti [28]. The quantity of N accumulated in the leaf lettuce was determined through the micro Kjeldahl method espoused by Kalra [23]. The nitrate content of the soil was determined colorimetrically following a method espoused by Jones [25] after extraction with 2 M potassium chloride solution. Briefly, the colorimetric measurement followed diluting the soil extracts and standards with 3.0 mL of distilled water. Then, 2.0 mL of antimony sulphate solution and 6.5 mL of chromotropic acid solution were added in quick succession to each tube. The contents were mixed and absorbance was read from the UV-Vis spectrophotometer at 420 nm after standing the mixture for 2 h. The proportion of the first season's remnant N that was contributed to the total amount of N absorbed into the leaf lettuce in the second season was calculated as;

$$\frac{\text{N accumulated in the lettuce in each of the treatment s/ control experiment}}{\text{N accumulated in the lettuce in the amended soils}} \times 100 \quad (1)$$

The microbial biomass C and N were determined by the fumigation-extraction method developed by Brookes et al. [29] and Wu et al. [30] by adopting 0.45 for the fraction of microbial biomass C (KC) and N (KN) mineralized as espoused by Zhang et al. [31]. Fumigation followed strict adherence to the procedure outlined by Zhang et al. [31]. Microbial biomass C was then quantified by wet digestion with potassium dichromate following the method outlined in Jones [25], whereas microbial biomass N was quantified with the CHN elemental analyzer (LECO, Truspec, St. Joseph, MI, USA). The soil used had the following characteristics; pH (CaCl$_2$) = 5.56 ± 0.21, Total N (%) = 0.12 ± 0.05, Ca (g/kg) = 1.064 ± 0.17, K (g/kg) = 0.09 ± 0.01, Mg (g/kg) = 0.06 ± 0.02, Olsen P (ppm) = 19.87 ± 2.89, B (ppm) = 6.72 ± 0.98, Mo (ppm) = 0.23 ± 0.07, Zn (ppm) = 43.59 ± 7.17. The chemical properties of the BM biochars and their corresponding NEBs fertilizers can be found in Luyima et al. [7]. The B, Mo, and Zn contents of BM 25 were 31.8 ± 2.26, 4.70 ± 0.77, and 568.47 ± 29.50, respectively, while those of BM 50 were 23.19 ± 1.99, 2.44 ± 0.82, and 549.13 ± 29.00, respectively. The textural composition of the soil used is given in Yoo et al. [32].

2.4. Statistical Analysis

All the data obtained was subjected to a one-way analysis of variance (ANOVA) using Microsoft Excel 16 data analysis ToolPak for the examination of significant differences amongst the treatments. The significantly different data was then subjected to a Tukey post hoc *t*-test with the *p*-value set at 5% using the studentized q tables to quantify the significant differences between treatments. Variability of the data was expressed as the

standard deviation and the statistical differences between treatments were exhibited with small letters of the English alphabet. For delineating whether Olsen P: Total P is dependent on the quantity of organic matter in the soil, a regression relationship was conducted.

3. Results

3.1. Dynamics of N and P in Both the Leaf Lettuce and Soil

As exhibited in Figure 1a, the amount of P accumulated into the leaf lettuce was highest in the TSP amendment followed by the BC treatment, while the lowest P accumulation resulted from BM 25 and BM 50 amendments. It is worthy to note, however, that while the quantity of P accumulated into the leaf lettuce decreased in the second season in the TSP-amended soil, all other treatments elicited increases for P accumulated into the leaf lettuce. P accumulated into the leaf lettuce decreased from 5.35 mg/g of plant in the first season to 5.15 mg/g of plant in the second season. On the other hand, P accumulated into lettuce increased from 4.29, 3.59, and 3.67 mg/g of plant in the first season to 4.34, 3.82, and 4.11 mg/g of plant in the second season in BC, BM 25, and BM 50 amendments, respectively.
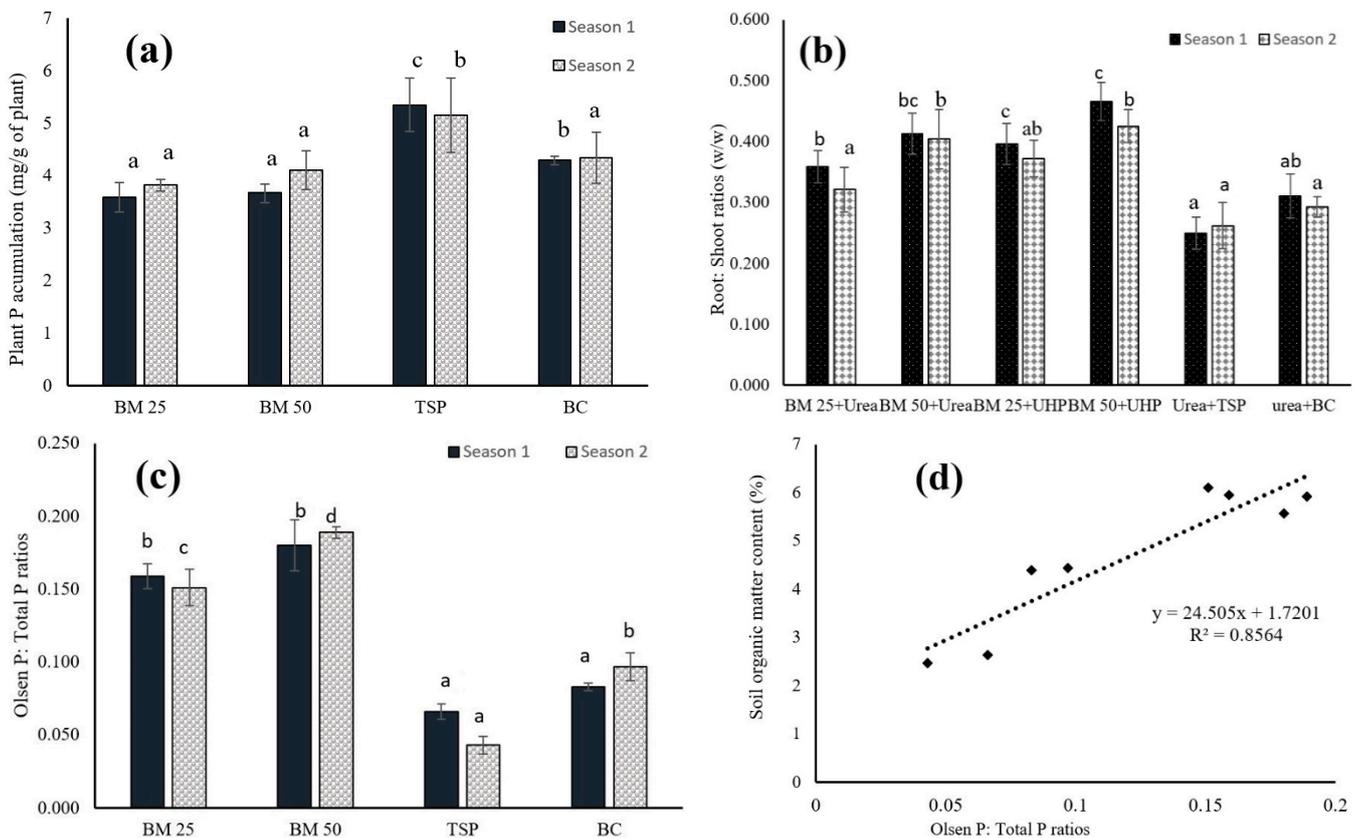

Figure 1. (**a**) Quantity of P accumulated into the leaf lettuce from the two seasons, (**b**) root: shoot ratios of the leaf lettuce produced with the different amendments, (**c**) Olsen P: total P ratios of the soil amended with the different P sources, (**d**) linear relationship between Olsen P: total P and soil organic matter content of the soil.

As shown in Figure 1b, the root: shoot ratio values were lowest for the lettuce grown in the conventional amendment of Urea+TSP in both seasons. The highest root to shoot ratios were obtained from the BM 50+UHP amendment, even though the values obtained in the second season were not statistically different from those got with the BM 50+Urea amendment. The root to shoot ratio values in all amendments except Urea+TSP (whose root to shoot ratios increased) decreased in the second season. Urea and UHP-enriched biochars elicited higher root to shoot ratios in the leaf lettuce than the rest of the amendments i.e., Urea+TSP and Urea+BC. The Olsen P to total P ratios were highest in the BM 50 treatment

in both seasons followed in a descending order by BM 25, BC, and TSP amendments. The ratios decreased in the second season in the BM 25 and TSP amendments, while those in the BM 50- and BC-amended soils increased as shown in Figure 1c. There was a strong linear relationship between the Olsen P to Total P ratios and the content of the soil organic matter as shown in Figure 1d.

With regards to plant N accumulation, NEBs amendments led to the production of leaf lettuce with the largest amounts of N with the lettuce produced from the BM 25+UHP treatment containing the highest quantities of it in both seasons as shown in Figure 2a. The lowest content of N was found in the lettuce grown on the Urea+TSP amendment followed by that produced with the Urea+BC treatment and unlike the rest of the amendments whose plant N content increased in the second season, it elicited a slight decrease in the lettuce N content in the second season. Similarly, the nitrate N content was highest in the soil amended with NEBs followed by the Urea+BC treatment, while the conventional amendment of Urea+TSP led to the lowest content of nitrates in the soil as shown in Figure 2b. In both seasons, the applications of UHP containing NEBs resulted in the highest content of remnant nitrates in the soil. The abundance of nitrates in soils amended with NEBs is most probably the reason why those soils contributed the largest percentage of the first season's remnant N to the N accumulated into the lettuce grown in the second season of the experiment as shown in Figure 2c. In that regard, BM 25+UHP amendment supplied the biggest portion of the first season's remnant N followed in a descending order by BM 50+UHP, BM 50+Urea, BM 25+Urea, Urea+BC, and Urea+TSP.
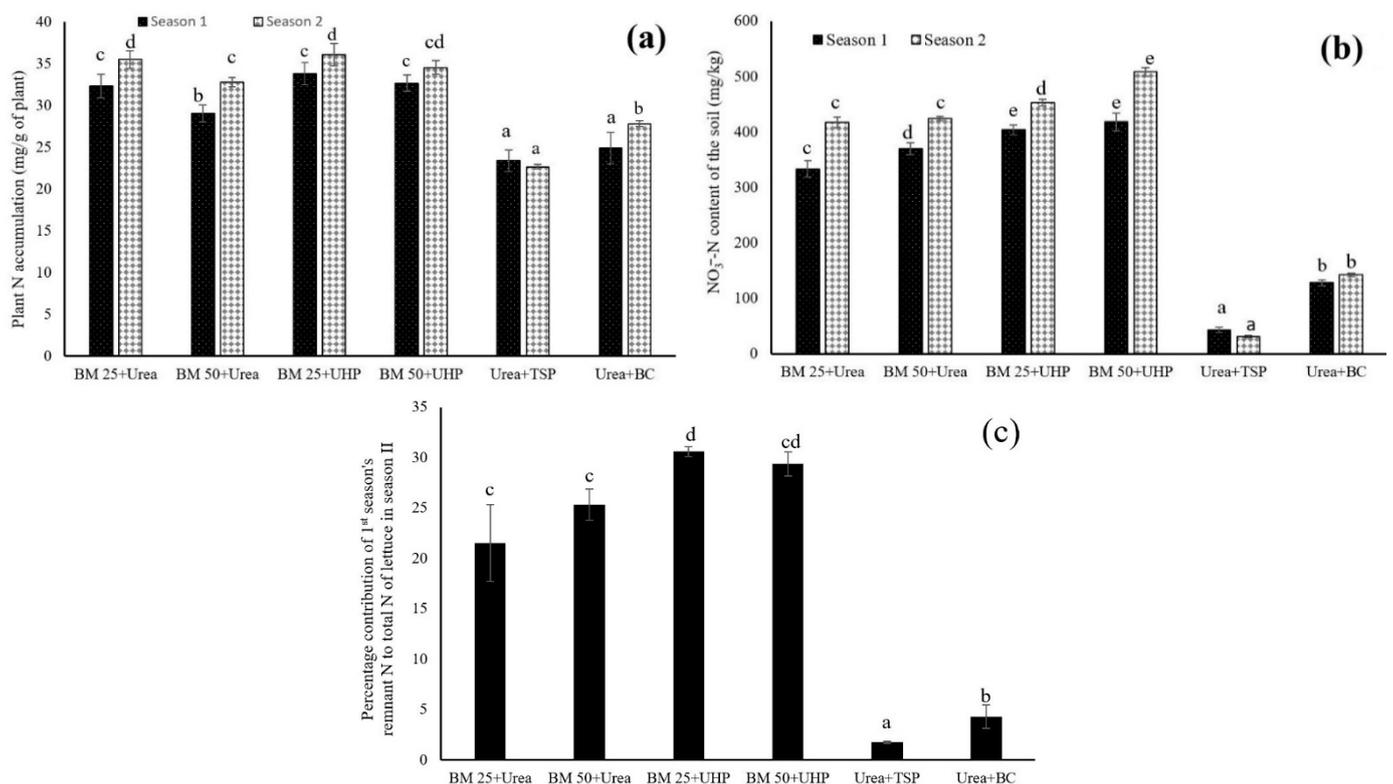

Figure 2. (**a**) The quantities of N accumulated into the leaf lettuce grown on the different amendments, (**b**) nitrate N contents of the soil under different amendments, (**c**) the percentage contribution of first season's remnant N to the total N accumulated into the leaf lettuce in the 2nd season.

3.2. Dynamics of Other Macronutrients and Micronutrients in Both the Leaf Lettuce and Soil

The quantity of potassium (K) accumulated into the leaf lettuce was highest in the Urea+TSP amendment followed in a descending order by the Urea+BC amendment, BM 50 and BM 25 containing NEBs as can be seen from Figure 3a. The Urea+TSP amendment

resulted in 39.39 and 37.97% more K accumulation into the leaf lettuce in the first and second seasons, respectively, than the Urea+BC amendment. In the same vein, Urea+BC amendment led to 28.30 and 20.46% more K accumulations in the first and second seasons, respectively, than the best performing NEBs amendments, which were BM25+Urea in the first season and BM50+UHP in the second season. The K accumulated into leaf lettuce was generally higher in the second season than in the first season. Ca accumulated into the leaf lettuce was lowest in the Urea+TSP amendment and highest in the Urea+BC amendment. The application of Urea+BC increased Ca accumulation in the leaf lettuce by 98.78 and 109.44% in the first and second seasons, respectively, in comparison to the conventional Urea+TSP amendment. There were no statistical differences in Ca accumulations between the Urea+BC amendment and the BM50+Urea in both seasons as well as BM 50+UHP in the first season, as shown in Figure 3b.
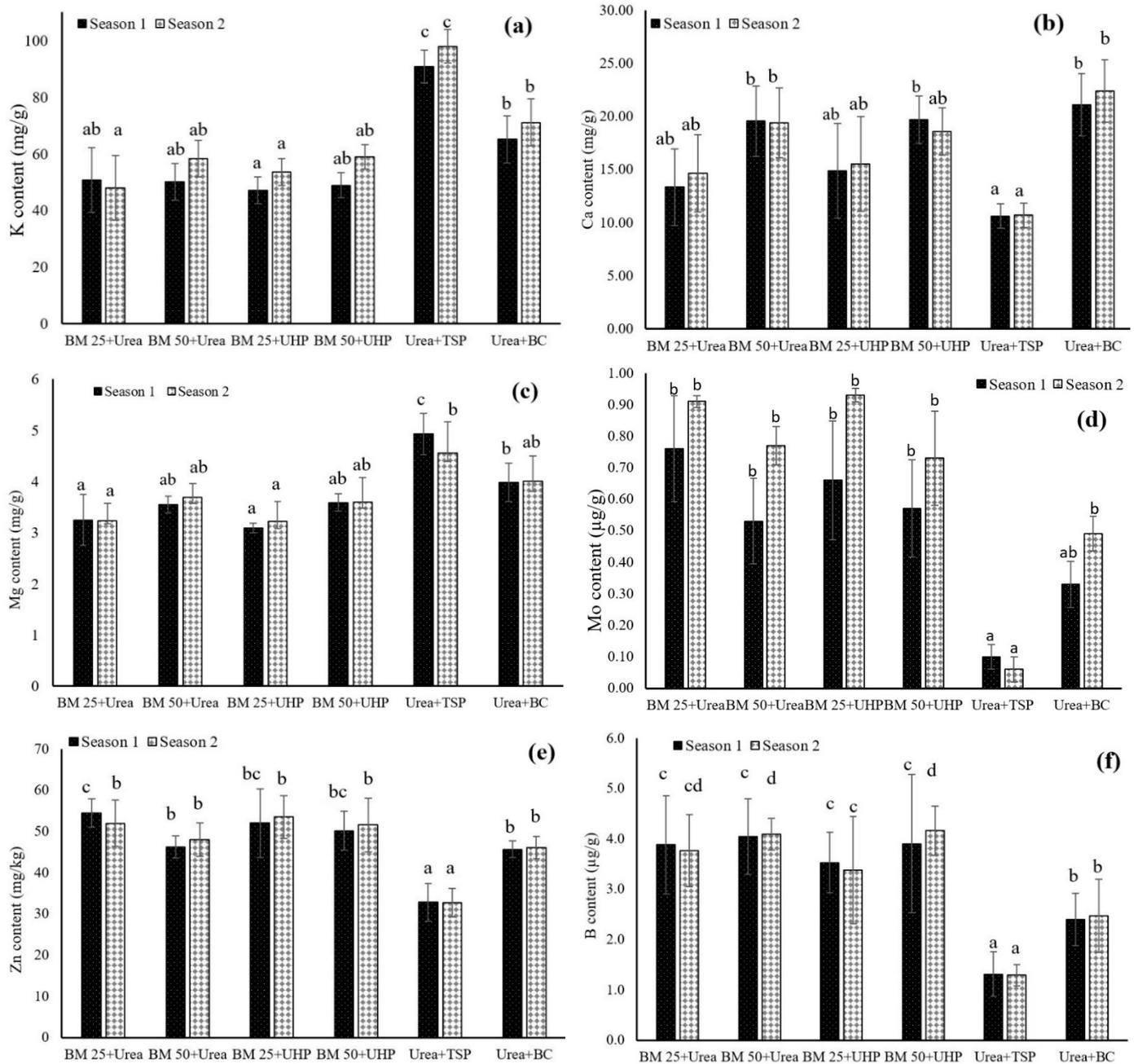

Figure 3. The quantities of (**a**) K, (**b**) Ca, (**c**) Mg, (**d**) Mo, (**e**) Zn, and (**f**) B accumulated into the leaf lettuce grown on the different amendments.

For magnesium (Mg) accumulation, the conventional amendment of Urea+TSP produced leaf lettuce with the highest content followed in a descending order by Urea+BC amendment, BM 50, and then BM 25 containing NEBs fertilizers. In comparison with the Urea+BC amendment, Urea+TSP amendment increased Mg accumulation by 23.72 and 13.81% in the first and second lettuce growing seasons, respectively. For the accumulation of all the other micronutrients studied, including Zn, B, and Mo into the leaf lettuce, the conventional amendment of Urea+TSP trailed both the NEBs and Urea+BC amendments. The leaf lettuce with the highest contents of these nutrients generally came from the NEBs fertilizers amendments followed by the Urea+BC treatment, even though there were no significant statistical differences between the former and the latter with regard to Zn and Mo accumulations. The worst performing NEBs fertilizer amendments improved Mo accumulation by 430.00 and 1116.67% in the first and second seasons, respectively, in comparison with the conventional amendment of Urea+TSP. For B, the worst performing NEBs fertilizer amendments improved its accumulation by 169.23 and 192.31% in the first and second seasons, respectively. Similarly, the worst performing NEBs amendments increased Zn accumulation into the leaf lettuce by 41.01 and 46.91% in the first and second seasons, respectively, in comparison with the conventional Urea+TSP amendment. For all the three micronutrient elements studied, i.e., Zn, B, and Mo, the Urea+BC amendment trailed NEBs fertilizer amendments as can be seen from Figure 3d–f.

The soil contents of available cationic nutrients of K, Ca, and Mg were ahighest in the Urea+BC amendment and lowest in the conventional amendment of Urea+TSP as shown in Figure 4a–c. In comparison with the conventional amendment, the Urea+BC amendments increased soil-available K by 120.00 and 221.82% at end of the first and second growing seasons, respectively. The soil-available Ca content increased by 167.64 and 184.76% in the first and second seasons, respectively, in the Urea+BC-amended soils in comparison to the conventional amendment. Additionally, the Urea+BC amendment increased soil-available Mg by 159.46 and 351.35% in the first and second growing seasons, respectively, in comparison to the conventional amendment of Urea+TSP. There were no significant statistical differences between the conventional amendment and the NEBs fertilizer amendments in the first season as far as soil-available K was concerned. The worst performing NEBs fertilizer amendments increased soil-available K by 6.15 and 54.54% in the first and second growing seasons, respectively, when compared with the conventional amendment. For soil-available Ca and Mg, the worst performing NEBs fertilizer amendments increased their contents by 72.55 and 94.59%, respectively after the first season in comparison to the conventional amendment of Urea+TSP treatment. At the end of the second season, the worst performing NEBs fertilizer amendments increased soil-available Ca and Mg by 80.95 and 263.64%, respectively in comparison to the Urea+TSP amendment.

For the contents of soil-available micronutrients of Zn, Mo, and B, the NEBs amendments outperformed both the Urea+BC and Urea+TSP amendments. The worst performing NEBs fertilizer amendments increased soil-available Zn by 374.31 and 508.61% in the first and second growing seasons, respectively, in comparison to the conventional amendment. There were no significant statistical differences between the Urea+BC and the conventional amendment of Urea+TSP in both seasons as can be seen in Figure 4d. with regards to soil-available B, the worst performing NEBs fertilizer amendments increased the contents by 199.81 and 215.70% at the end of the first and second seasons, respectively, in comparison to the conventional amendment of Urea+TSP (see Figure 4e). On the other hand, the worst performing co-pyrolyzed biochars increased soil-available molybdenum content by 550.00 and 615.38% in the first and second seasons, respectively, as compared to the conventional amendment of Urea+TSP (see Figure 4f). The amounts of soil-available micronutrient elements generally increased in the second season and except for the soil-available Zn, the Urea+BC amendment led to accumulation of higher quantities of soil-available B and Mo than in the Urea+TSP amendment in both growing seasons.
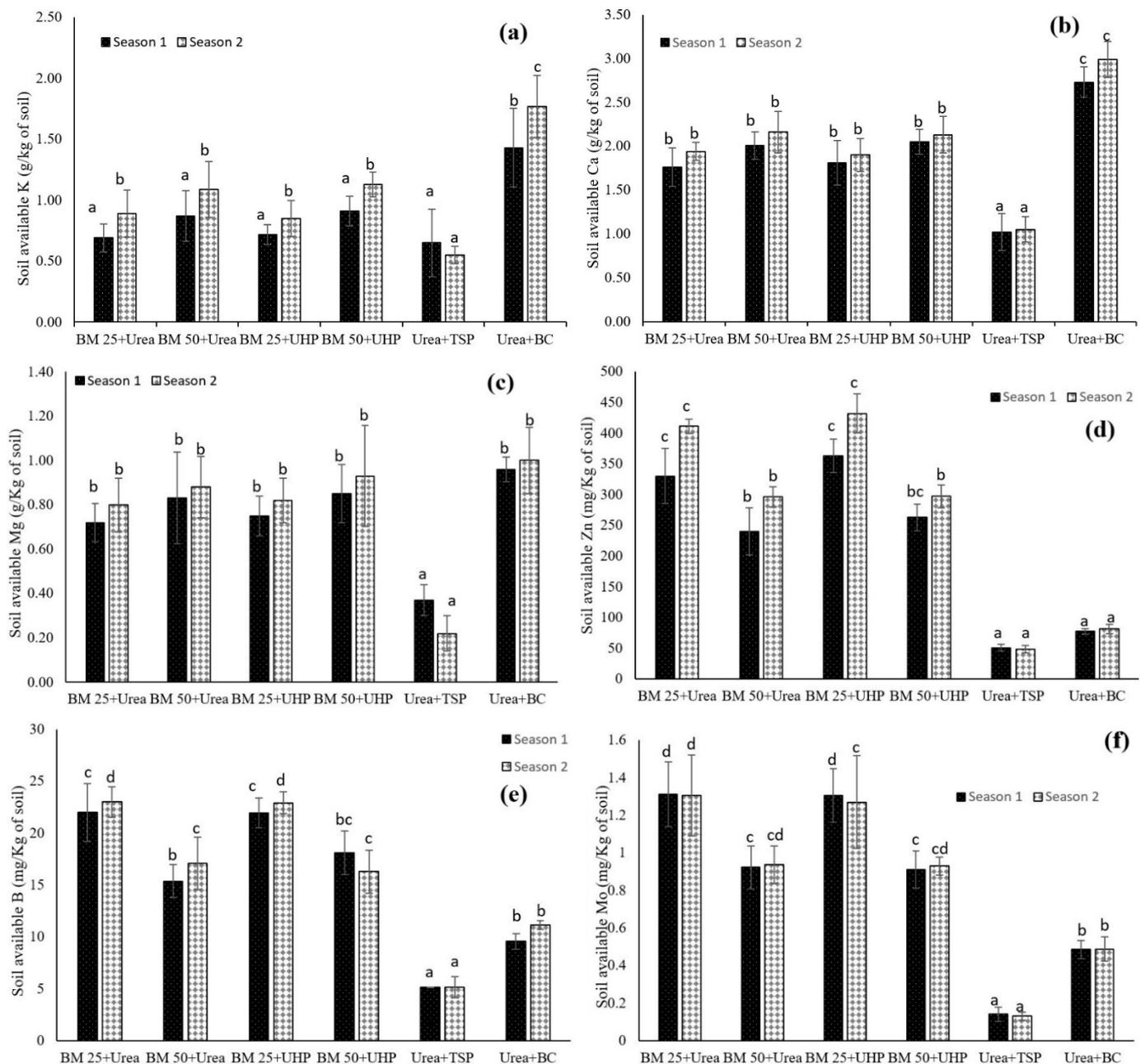
Figure 4. The quantities of soil-available (**a**) K, (**b**) Ca, (**c**) Mg, (**d**) Zn, (**e**) B, and (**f**) Mo obtained from the different amendments at the end of each lettuce growing season.

3.3. Dynamics of Soil Microbial Biomass C, N, and C:N Ratios

All the amendments with pyrolyzed materials increased the microbial biomass C content with the highest increments produced by the urea containing NEBs biochar amendments as shown in Figure 5a. The NEBs biochar fertilizers containing 25% bone char (BM 25 NEBs), i.e., BM 25-Urea and BM 25-UHP caused the highest increases in microbial biomass C. This was followed in the descending order by the NEBs biochars containing 50% bone char (BM 50 NEBs), Urea+TSP, and Urea+TSP amendments. The worst performing BM 25 NEBs amendments increased microbial biomass C by 75.60 and 76.22% in the first and second growing seasons, respectively, in comparison to the conventional amendment of Urea+TSP (see Figure 5a). Additionally, as compared to the conventional amendment, the worst performing BM 50 NEBs amendments increased microbial biomass C by 37.38 and 31.60% in the first and second growing seasons, respectively (see Figure 5a). BM 25 NEBs

caused higher increments in microbial biomass C content than the BM 50 NEBs. Although the Urea+BC amendment increased microbial carbon content by 15.99 and 21.50% in the first and second growing seasons, respectively, in comparison to the conventional amendment, there were no significant statistical differences between the two treatments in the first season (see Figure 5a).
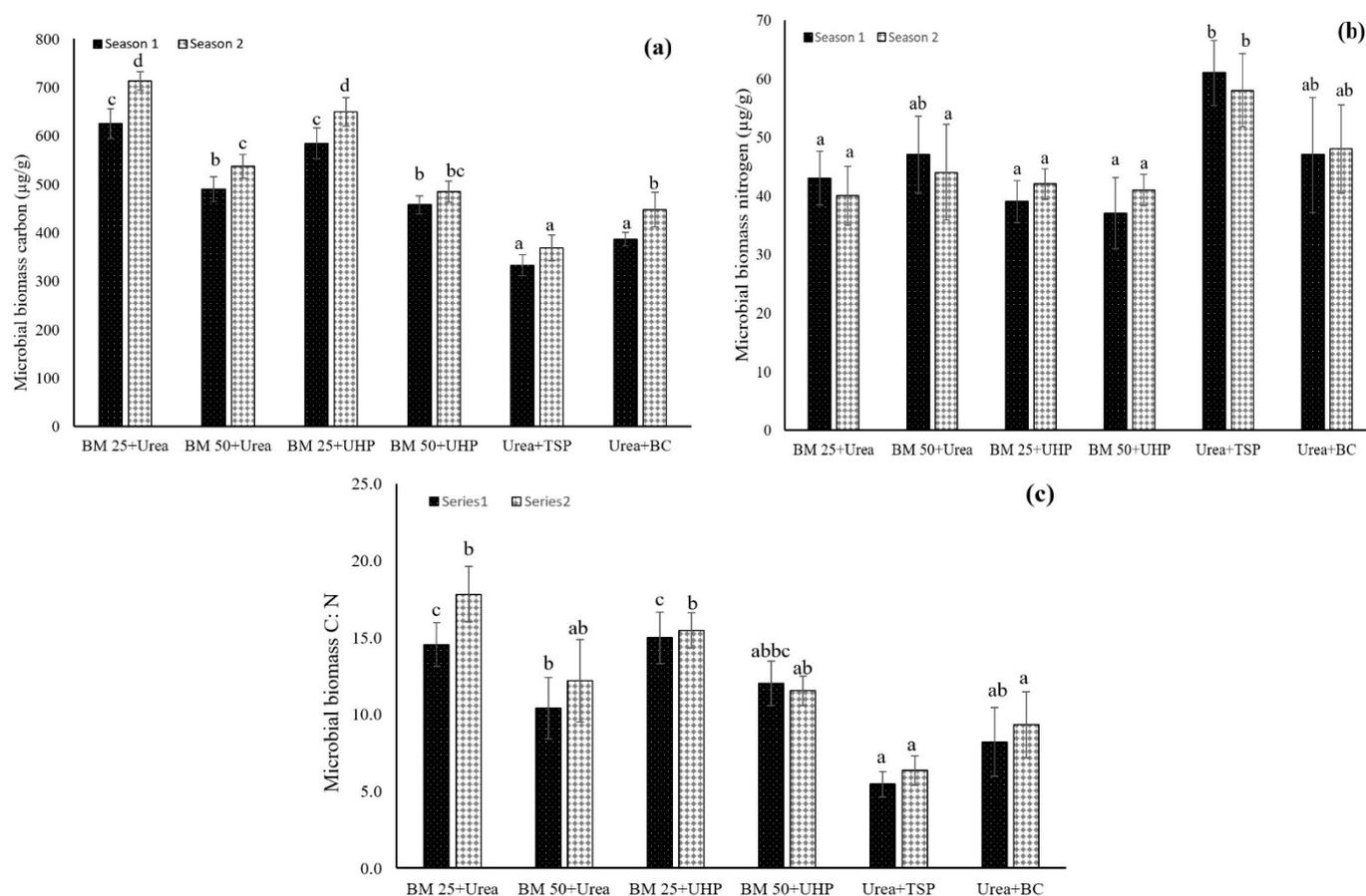

Figure 5. Microbial biomass (**a**) C, (**b**) N, and (**c**) C: N ratios of the soil under different amendments at the end of each lettuce growing season.

Contrary to the above-mentioned observations, the conventional amendment of Urea+TSP caused higher contents of soil microbial biomass nitrogen than the amendments of pyrolyzed materials as shown in Figure 5b. The conventional amendment increased soil microbial N content by 29.79 and 31.82% in the first and second growing seasons, respectively, as compared to the best performing NEBs amendments (see Figure 5b). In the same vein, the conventional amendment elicited 29.79 and 20.83% more microbial biomass N than the Urea+BC amendment in the first and second growing seasons, respectively. The quantity of microbial biomass N contained in the Urea+BC-amended soils was not significantly different from the microbial biomass N found in both the NEBs fertilizer-amended soils and the soil amended with Urea+TSP as can be seen in Figure 5b.

In both seasons, the NEBs-amended soils registered the highest quantities of microbial biomass C:N ratios, while the lowest values came from the soil treated with the conventional amendments of Urea+TSP as shown in Figure 5c. The BM 25 NEBs-amended soil registered the lowest microbial biomass C:N ratios contained 62.45 and 58.93% more C:N than the conventional amendment of Urea+TSP after the first and second lettuce growing seasons, respectively, as can be seen from Figure 5c. The BM 50 NEBs-amended soil that contained the lowest microbial biomass C:N actually had 47.60 and 44.97% more microbial C:N than the conventional amendment of Urea+TSP after the first and second growing seasons,

respectively. Additionally, the Urea+BC-amended soils contained 33.50 and 31.94% more microbial biomass C:N ratios than the Urea+TSP amendment after the first and second lettuce growing seasons, respectively. It should be noted, however, that there were no significant statistical differences in the microbial biomass C:N of both the Urea+BC and Urea+TSP amendments after the second lettuce growing season.

4. Discussions

4.1. Dynamics of Nitrogen and Phosphorus in Both the Leaf Lettuce and Soil

The leaf lettuce grown on the Urea+TSP amendment accumulated more P than the one grown on other amendments because P in TSP is more readily available than that of BC. This observation concurred with one made by Siebers et al. [33], who discerned that a mineral P fertilizer in the form of $Ca(H_2PO_4)_2$ outperformed BC in the supply of P to potatoes, wheat, and onions. Later on, a study by Zwetsloot et al. [34] supported that observation by indicating that maize accumulated more P from TSP than from BC and co-pyrolyzed BC and wood biochar. In the present study, the pure BC amendment led to more P accumulation in the leaf lettuce than both BM 25 and BM 50 amendments because co-pyrolysis further slows down P release [7,13,35]. The slow release of P from BC, BM 25, and BM 50 might explain the higher root to shoot ratios than those obtained with the conventional TSP fertilizer. Indeed, studies by Ismail et al. [36] and Li et al. [37] indicated that P deficiency induced a preferential proliferation of roots over shoots as an adaptive mechanism to stress brought about by low P availability. Later on, a study by Wacker-Fester et al. [38] exhibited that the P content of potatoes negatively correlated with the root to shoot ratios.

Despite their poor performance as suppliers of P to the growing lettuce, BM 25, and BM 50 P amendments resulted in higher reservoirs of P after every growing season than either TSP or BC, as evidenced from the high Olsen: total P ratio values of the soils amended with them. This most likely ensued from the fact that TSP is highly water-soluble and thus, its availability to plant roots dwindles over time through adsorption reactions with mineral oxide surfaces as Kucey et al. [39] indicated. As Øgaard [40] indicated, Olsen P to total P ratios of the soil are an important measure of the soil's capacity to bind P in sparingly soluble forms and of the capability for plants to utilize the added P fertilizers. The study found strong negative correlations between the Olsen P to total P ratios and clay, as well as iron contents of the soil. Based on the observations made in the above-mentioned study, it can be deduced that the co-pyrolyzed BC and cow dung biochar amendments of BM 25 and BM 50 increased the availability of P compared with both BC and TSP. The high r^2 value (0.8564) of the linear relationship obtained between the Olsen P to total P ratios and soil organic matter content accords well with the results obtained by Shen et al. [41]. This implies that the increased availability of P observed with the BM 25 and BM 50 amendments partly ensued from the increment in organic matter caused by those amendments. Indeed, BM 25 and BM 50 P amendments contained higher contents of carbon than both the BC and TSP as shown by Luyima et al. [7].

The observed higher accumulations of N from the NEBs amendments than from the Urea+TSP and Urea+BC amendments were in agreement with several studies, for example by Utomo et al. [42], who indicated that the biochar-enriched with either ammonium sulphate or nitric acid led to more N accumulation in rice than the conventional amendment. Shi et al. [43], who found that the urea-enriched biochar increased the amount of N accumulated into maize later supported that observation. In the same vein, Dietrich et al. [44] found that biochar loaded with digestates increased N accumulation and doubled the biomass production in young maize plants. Several reasons have been given for the increased N accumulation in plants upon N-enriched biochar application. The enhancement in N accumulation by crops from the enriched biochar amendments can be ascribed to the reductions in NO_3 leaching, NH_3 volatilization, and N_2O denitrification [21]. These reductions in turn arise from strong adsorptions of NH_4^+, NH_3, and NO_3^- on to the biochar

surfaces [45]. The end result of these adsorptions is the release of N over an extended period of time in quantities commensurate with the crop requirements.

Indeed, the ability of the biochar-based slow release fertilizers to reduce leaching of NO_3^- is confirmed by the higher concentrations of NO_3^- found in the NEBs fertilizer amendments than those obtained from other amendments. That observation concurred with the observations made by Haider et al. [46], Hagemann et al. [47], and others. Hagemann et al. [47] showed that 70 and 58% of the total nitrate adsorbed by the biochar from the soil and compost, respectively, was released slowly. Haider et al. [46] observed that the concentrations of NO_3^- were higher in the upper 15 cm layer of the soil in the biochar-amended soils than in the control. Additionally, the control contained more NO_3^- in the subsoil than the biochar-amended soils and the amount of NO_3^- found in the subsoil reduced with the increasing biochar application rates. Despite the higher quantities of NO_3^- in the top soil, there was no discernible effect on the maize yield over a four-year period of study. In the current study, it was found that N, which remained in the NEBs-amended soils in the first season, contributed between 21.54–30.61% to the total amount of N accumulated in the leaf lettuce in the second growing season. This can be ascribed to the attenuation of leaching of N by the nutrient-enriched biochars, as studies by Shi et al. [43], Luyima et al. [7] and others have confirmed. This observation indicates the ability of the NEBs amendments to conserve applied N resources beyond a single growing season.

4.2. Dynamics of Other Macronutrients and Micronutrients in Both the Leaf Lettuce and Soil

Apart from K and Mg contents, which were higher in the conventional Urea+TSP amendment, the rest of the nutrient elements studied were highest in the leaf lettuce grown with charred amendments, especially NEBs fertilizers that produced leaf lettuce with the highest contents of Zn, B, and Mo, while Ca was highest in the Urea+BC amendment. While studies into the effects of nutrient-enriched biochars on the dynamics of nutrients of importance to human health in both crops and soil are scanty, several experiments have discerned the effects of different biochar types on nutrient availability both in soil and to crops. Such studies include the one by Deenik et al. [48], who assessed the effects of charcoal on nutrient uptake and concentration in the consumable parts of the leaf lettuce. The study found that charcoal amendments with or without fertilizers increased contents of Ca, Mg, and Zn in the leaf lettuce, while the increases in the uptake of those nutrient elements were only observed in the combined charcoal and fertilizer amendments. This observation is in tandem with what we observed in our study concerning Ca, Mg, and Zn as both the NEBs and Urea+BC amendments increased the contents of those nutrient elements in the leaf lettuce (Figure 3b,c,e).

A study by O'Toole et al. [49] found that wheat straw biochar produced in the Kilns increased both K and Zn contents in rye grass, but the contents of Ca and Mg reduced in comparison to the control. They attributed the observed decreases in Ca and Mg contents to the high amounts of Zn in the biochar, which might have competed with them on the absorption sites in the soil. Surprisingly, both our biochar and BC contained high amounts of Zn, but it never interfered with the absorptions of other nutrient elements, since the leaf lettuce grown with both NEBs fertilizers and BC contained higher quantities of all the nutrient elements except K and Mg in comparison to the conventional amendment. The high Zn contents of both the co-pyrolyzed biochar and BC might have ensued from the contamination by the galvanized metal containers used for charring, as O'Toole et al. [49] suggested. Additionally, Zn is usually used as a supplement in animal feeds [50], and indeed, Olszyk et al. [51] found high Zn concentrations (5000 to 6800 ppm) in their swine solids biochar even though the Zn content of the lettuce leaves was not increased upon that biochar application. However, Gartler et al. [52] found no statistically significant differences between the zinc contents of the leaf lettuce grown on the control and biochar-amended soils, despite the fact that the amount of zinc in the leaf lettuce grown on the latter were higher than those of the control experiment.

Gunes et al. [53] studied the effects of P-enriched poultry manure biochar on mineral composition of leaf lettuce and found that the biochar amendments increased the concentrations of P and K in the leaf lettuce, but decreased Ca, Mg, and Zn contents in comparison to the control. The study also discerned that poultry manure biochar without P enrichment had no effect on Ca and Mg contents of leaf lettuce. The observations regarding Ca, Mg, and Zn contravene the results of our study possibly due to differences in soil pH, because Gunes et al. [53] applied biochar to an alkaline soil and the solubility of those micronutrients is generally low in such soils. Contrary to what Gunes et al. [53] observed, Rees et al. [54] reported increments in Zn content in the Cd-Zn hyper accumulator plant called Noccaea caerulescens grown on alkaline soils amended with biochar, whist the amendment on acidic soils reduced Zn accumulation. Rees et al. [54] employed heavy metals-contaminated soils and the decrease in leaf Zn content was probably due to an increase in soil pH with addition of biochar, especially for a strongly acidic soil [51]. It should be noted that our soil was not strongly acid (CaCl$_2$ pH of 5.7), which may explain why the NEBs fertilizers and BC elicited increases in soil pH actually led to increases in Zn contents in the leaf lettuce. Secondly, the pH changes were not very large as the pH of NEBs fertilizers-amended soils ranged between 6.31–6.54 and 6.63–6.79 in the first and second seasons, respectively.

Syuhada et al. [55] suggested that reductions in maize leaf Ca and Mg concentrations caused by biochar application to a fertilized sandy Pozol were due to growth dilution. The study, however, found increments in K concentration of the leaves of maize grown on biochar-amended soils. In agreement with our study, Sorrenti et al. [56] reported an increase in leaf K when tree wood biochar was applied to kiwifruit vines. However, the study contravened our observation with Zn as the wood biochar decreased the Zn content of the vines. Similarly, Ramzani et al. [57] found increases in essential nutrients in the quinoa seeds with sulphur-acidified biochar. Olszyk et al. [51] found that animal manure-based biochars decreased Ca, Mg, and Zn contents in the leaves of lettuce and carrot taproots even though the decreases were smaller in the latter than in the former. The increments registered for all the micronutrients of the leaf lettuce grown under the amendments of pyrolyzed materials might have been due to three main reasons namely; direct supply of nutrients, changes in soil pH and reduction of the dilution effect. For soil-available nutrients, both NEBs and BC amendments contained higher contents than the conventional treatment of Urea+TSP. There have been a few studies concerning the effects of nutrient-enriched biochar amendments on the dynamics of the soil macro, meso, and micro nutrient elements. For example, a study by Gaskin et al. [58] found that the peanut hull biochar increased soil-available K, Ca, and Mg, while pine chip biochar decreased the contents of soil-available Ca, but had no effect on other soil nutrient elements. However, the changes in the soil-available nutrients did not reflect in the maize tissue except for K in the peanut hull biochar-amended soil in the 2006 season only. The results obtained from the present study accord well with what was observed in the peanut hull biochar amendments since both the NEBs fertilizers and Urea+BC amendments resulted in higher contents of soil-available K, Ca, and Mg than the conventional amendment. Additionally, our observations regarding soil-available Zn were in agreement with what Moradi et al. [59] found when working with a saline soil because a nutrient-enriched biochar increased Zn content of the soil. Another study by Kizito et al. [60] enriched biochar with the nutrients from anaerobic digestate indicated that the enriched biochar increased the amounts of K and Ca in the soil, but decreased Zn content.

4.3. Dynamics of Soil Microbial Biomass Carbon, Nitrogen, and C:N Ratios

The microbes residing in the soil are a sensitive indicator of soil quality as well as a bio-indicator of soil ecosystem sustainability [61]. While there is a paucity of data regarding the effects of nutrient-enriched biochar amendments on soil microbes, several studies have assessed the influence of biochar on several soil microbiological parameters, although the results are quite inconsistent. An early study by Castaldi et al. [62] indicated that

biochar amendment to an acidic soil did not have any significant impact on the microbial biomass C after both 3 and 14 months from incorporation into the soil. However, a 21-day incubation study by Zavalloni et al. [63] revealed that biochar amendments enhanced the microbial biomass C, but generally decreased the microbial biomass N content. In another similar incubation study, Luo et al. [64] found that biochar significantly increased microbial biomass C in both acidic and alkaline soils amended with biochar. However, the quantity of microbial biomass C obtained after 180 days of incubation was lower than that obtained after 90 days.

In a long-term field study, Zhang et al. [31] examined the impact of consecutive application of biochar over a four-year period on microbial biomass C, N, and C/N ratio and found that the microbial biomass C and C/N ratios increased with increasing biochar application rates. The study, however, indicated that the effects of biochar on microbial biomass N were inconsistent, as the low application rate of biochar (4.5 tons per hectare) decreased it while the high application rate (9 tons per hectare) increased it. In another long-term field study, Du et al. [65] indicated that application of 1% peanut-shell biochar to a fluvo-aquic soil increased microbial populations, microbial biomass, and actinomycetes. Similarly, Zheng et al. [66] indicated that a single biochar application to a paddy field increased microbial biomass carbon at the end of the fourth year of the experiment. In the present study, NEBs fertilizers and BC amendments to the sandy soil resulted in higher microbial biomass C and C/N ratios, but lower microbial biomass N compared to the conventional Urea+TSP amendment and thus, these observations concurred with most of the above-cited studies.

Zhang et al. [31] proclaimed that changes in microbial biomass C signify microbial growth, death, and organic matter degradation. The higher microbial biomass C content of the NEBs fertilizers-amended soils than those observed in the rest of the amendments in this study indicate that the NEBs amendments enhanced microbial growth more than the rest of the amendments. The lower microbial biomass N obtained with the NEBs amendments than with the rest of the amendments indicates that N supply from the NEBs fertilizers to the soil microbes was the lowest of all the amendments, which confirms the N slow-release potentials of the NEBs fertilizers. Variations in microbial biomass C:N ratios are indicative of the relative availability of C and N to soil microbes, but can also signify changes in the structures of the soil microbial community [31]. While it is unclear if the increase in the microbial C:N observed in our study was due to changes in microbial community structure, one clear thing to note is that the changes ensued from N immobilization by the NEBs fertilizers. It can be deduced, therefore, that nitrogen-enriched biochars act more as carbon sources than nitrogen sources for the soil microorganisms.

5. Conclusions

The nutrient-enriched biochars increased the amounts of nutrient elements accumulated in the consumable parts of the leaf lettuce, but also enhanced the availability of those nutrients in the soil. This might have ensued from the fact that the nutrient-enriched biochar amendments employed in the present study were rich in the meso and micro nutrient elements. However, these changes might also have resulted from the changes in soil properties caused by the applied nutrient-enriched biochars, even though that area is out of the scope of the current study. The results obtained in this study indicate that nutrient-enriched biochars can biofortify crops with essential nutrients, which is vital in producing high-quality nutritious food. Secondly, these observations show that charred bone waste is a novel and renewable source of P, which can replace the conventional TSP in vegetable production. It is important to note that more studies involving different biochar types used for enrichment should be undertaken to discern their effects on the nutrient dynamics. This is because several studies with biochar in this field have yielded inconsistent results depending on the biochar's biomass feedstock.

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