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Impact of Different Methods of Root-Zone Application of Biochar-Based Fertilizers on Young Cocoa Plants: Insights from a Pot-Trial

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Abstract: Effective and efficient nutrient management is central to best-practice agriculture, facilitating sustainable intensification while reducing negative externalities. The application of biochar-based fertilizers (BBF) in tropical agronomy has the potential to improve nutrient management by enhancing nutrient availability and uptake. Here, we performed pot-trials with *Theobroma cacao* L. seedlings planted in an Oxisol with critically low phosphorus levels. Four fertilizer levels were deployed, including BBFs using micro-dosed biochar (16 g plant^{-1} i.e., 0.3% soil amendment *w/w*) charged with mineral fertilizer. Three different fertilizer-placement levels (topsoil, root-zone hotspot and root-zone layer) were evaluated. The results from the topsoil application of mineral fertilizer (farmer practice) served as the reference data. The root-zone layer application of BBF increased the aboveground biomass, total leaf area and chlorophyll content index by 56%, 222%, and 140% respectively. Foliar phosphorus levels were also significantly elevated by 53%. The N:P ratio of the foliar tissue was improved, indicating the potential of BBF to ameliorate P limitations. Thus, low dosages of biochar, which is upgraded to BBF, can considerably improve plant nutrition. Small scale technology to produce biochar can be easily adopted and integrated in *T. cacao* systems. We suggest that BBF production and application within tropical, perennial systems can contribute to achieving a range of sustainable development goals (SDGs), including climate action.

Keywords: biochar-based fertilizer; biochar micro-dosing; rootzone-application; nutrient availability; phosphorus limitations; tropical carbon farming; climate change mitigation and adaptation; agroforestry; carbon-sink fertilizer

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1. Introduction

The agricultural sector is inherently vulnerable to climate variability and change and so are the livelihoods of people, especially farmers and rural populations in many tropical countries [1–3]. Thus, options for climate change adaptation and mitigation in agriculture are urgently needed. At the same time, sustainable agricultural intensification is required [4,5] to feed 10 billion people on this planet by 2055 [6]. The integration of biochar (BC) into tropical agricultural systems holds the potential to target these three major challenges in a combined approach.

Biochar is a recalcitrant and carbon-rich material obtained when exposing biomass to temperatures above 400 °C in a low- or no oxygen environment, i.e., through the process of pyrolysis [7,8]. Biochar shows a low bulk density, high porosity, high specific-surface area, a high cation exchange capacity and water holding capacity, and is inherently alkaline [9]. Thus, the application of BC as a soil amendment in agriculture holds the potential for climate change adaptation, e.g., by alleviating drought stress [10], due to its ability to directly increase soil porosity and water holding capacity [11]. Moreover, biomass pyrolysis and the non-oxidative use of the biochar obtained is considered a negative emissions technology, called Pyrogenic Carbon Capture and Storage (PyCCS), as biochar is rather recalcitrant in the environment and thus a form of technical, long-term carbon sequestration [12].

Biochar has proven to be effective in enhancing the fertility of degraded or highly weathered (tropical) soils [9,13–15]. This is directly mirrored in frequently reported increases in biomass production and/or crop yield, especially in, but not limited to, the tropics [16,17]. Jeffery and colleagues concluded in a meta-analysis that tropical yields increase by a mean of 25% after BC application [16]. Yet, these empirical findings vary strongly between systems and locations, and are determined by the complex BC × soil × crop interactions. In this context, recent meta-analysis revealed that the success of biochar application regarding yield increases depends on the combined application of BC with fertilizers, resulting in nutrient charged biochar i.e., biochar-based fertilization (BBF) [17]. Still, yield increase is induced by soil-physical and hydrological improvements, but also by altered soil chemistry, mainly by an elevation of the pH and thereby increasing plant nutrient availability [18,19].

In tropical Oxisols (highly weathered soils with low pH and rich in iron (Fe), and aluminum (Al) oxides.), the essential macro-nutrient phosphorus (P) is most often the limiting factor due to its immobilization by Fe and Al, as indicated by biomass N:P ratios of >16 [20]. Here, biochar may improve P availability due to its alkalinity and adsorption induced immobilization of aluminum [21,22]. It may also improve nutrient uptake by increasing in root biomass and surface area [23]. As with previous biochar research in the tropics focused on the application of pure biochar, there is limited knowledge on the effects of BBF in this context.

The worldwide cocoa production grew since 1980–1981 from 1.7 Mt to 5.5 Mt in 2019, globally covering 12.2 million ha, with Ivory Coast, Ghana and Indonesia being the main producers [24]. Due to their major spatial scale and production volumes, cocoa-based perennial (agroforestry) systems hold a high impact potential on world climate and food markets. Yet, they are also very vulnerable to climate change and significant decreases in suitable production areas have been projected for 2050 [25,26]. Additionally, aluminum toxicity and the uptake of cadmium is problematic for the sustainable cultivation of cocoa and its food safety [27,28]. Biochar was identified to be capable of reducing aluminum availability and associated nutrient stresses [19] and could furthermore contribute to the immobilization of cadmium [27]. An optimized nutrient management based on BBF, which increases availability and reduces the leaching of macronutrients, while reducing the mobility, accumulation, and consequential toxicity of contaminants, may be key to the sustainable intensification of the cocoa sector.

Past research on biochar amendment for cocoa seedlings observed enhanced nutrient availability and increased growth of seedlings being subject to BC application [29,30]. However, so far, only relatively high BC application rates (>2 t ha^{-1}) were investigated, which often represent amounts that are neither economically feasible nor ecologically sound, as the necessary feedstock would exceed the amount of biogenic residues that can be harvested within the same cocoa cultivation system. The BC of BBF application mode often observed in past biochar research resembles deployment in nursery-polybags or even distribution on the soil surface with consecutive incorporation (e.g., [31]), thus leaving a general knowledge gap on precision root-zone applications.

Here, we focus on root-zone applications of BBF. Instead of spreading the biochar, it is applied in the planting hole when the seedlings are transplanted from the nursery to the field. Thus, the soil does not come into contact with biochar over large areas of the field; however, a locally high concentration is present in the immediate root zone of the young plants, which can facilitate substantial yield increases even in rather fertile soils under temperate to alpine climates [32]. Root zone applications can therefore contribute to a more efficient deployment of BBF, yet to the best of our knowledge, it has not been tested in cocoa. It is therefore highly relevant to test whether benefits can be achieved through feasible application rates of biochar or BBF to the root-zone of cocoa systems. Here, we present data obtained from a greenhouse trial with a two-factorial design focusing on the interaction of fertilizer type (only BC, only mineral fertilizer, BBF–BC charged with mineral fertilizer, fermented BBF–lactic-fermented BC charged with mineral fertilizer) and type of application (homogeneous topsoil, root-zone hotspot, root-zone layer). To determine the potential of BC to enhance nutrient availability in cocoa systems, morphological and physiological characteristics, as well as the foliar stoichiometry of *Theobroma cacao* L., were examined during its seedling stage. Foliar nutrient concentrations, such as N%, P% or an N:P ratio can reflect soil nutrient availability [33,34].

Using this setup, we tested whether BBF promotes plant growth through enhanced nutrient availability beyond the effect of individual applications of mineral fertilizer or biochar and if fertilizer placement further determines the effect size.

2. Materials and Methods

2.1. Seeds, Soil and Biochar

Theobroma cacao L. ssp. forastero beans were provided by tropical greenhouses of the Division TROPAGS at the University of Göttingen, Germany. The beans were planted 6 weeks prior to the start of the experiment without stratification, in 6 cm × 6 cm × 12 cm nursery pods for germination. The "relict Oxisol" was obtained from the Taunus region (Germany) and was stored air-dried. This soil has a low P availability (<1 mg 100 g^{-1}) and high levels of Al (153 g kg^{-1}, Table 1 and Table S1). Biochar was obtained from a commercial pyrolysis unit (Abfallwirtschaft und Stadtreinigung Freiburg GmbH (ASF), Freiburg, Germany) that is certified according to the European Biochar Certificate (class EBC-Feed) [35]. BC was produced from landscaping wood by slow pyrolysis (Pyreg GmbH, Dörth, Germany) at temperatures of about 700 °C and was analyzed by Eurofins Umwelt Ost GmbH (Bobritzsch-Hilbersdorf, Germany, (Table 1 and Table S2) according to the EBC guidelines [35]. The biochar was highly alkaline (pH 10.1) and milled in a hammer mill equipped with a 3 mm sieve (CF420, 7.5–11 KW, Evertec, Beerfelden, Germany).

Table 1. Relict Oxisol and biochar used in this study: Content of total organic and total inorganic carbon (Corg/TIC), total nitrogen (N), ash, available P (Soil method: VDLUFA 6.2.1.1 via CAL extraction; Biochar method: DIN EN ISO 11885 (E22):2009-09 via borat extraction), total iron and aluminum (Fe/Al), pH, water holding capacity (WHC), cation exchange capacity (CEC), specific surface area determined by nitrogen gas adsorption (BET) and molar H/C_{org} ratio. Trace metal content of biochar is displayed in Table S2.

Parameter	Relict Oxisol	ASF Biochar
C_{org} [%]	0.3	84.4
TIC [%]	<0.1	0.9
N-min [%]	0.003	0.5
Ash 550 °C [%]	n.a. [1]	8.0
available P [%]	<0.001	2.3
Fe [%]	30.5	1.8
Al [%]	15.3	n.a. [1]
pH	5.9	10.1
WHC [%]	69	n.a. [1]
CEC [cmol kg^{-1}]	6.4	n.a. [1]
BET [m^2 g^{-1}]	n.a. [1]	179
H/C_{org}	n.a. [1]	0.14

[1] Not applicable.

2.2. Preparation of Biochar-Based Fertilizers

BBFs were prepared individually for each pot. Fertilizer dosages were calculated in accordance with the fertilizer recommendations for young cocoa systems [36] and included 40 kg P_2O_5 ha^{-1}. As valid for perennial systems, a concentrated application in a 1 m radius around the plants, at 1111 plants ha^{-1} (3 m × 3 m grid), was assumed. Considering the pot surface area, as a fraction of this fertilized area, results in application rates of 1.27 mL commercial liquid fertilizer (WUXAL Top P, WUXAL, Düsseldorf, Germany; NPK ratio of 5:20:5) per pot. The BBF was created by mixing the dry matter (DM) equivalent of 16.25 g BC, pristine or fermented, with 1.27 mL WUXAL (Düsseldorf, Germany) and 150 mL deionized water in a glass vessel, 24 h prior to soil application. During this 24 h adsorption period, the suspension was stored in the dark and shaken manually every 6 h. For the fermented BBF, BC and fresh foliar tree and grass biomass obtained from the greenhouse/the public yard next to the greenhouse were mixed in a 1:1 volume ratio and submerged in 3% saccharose solution (refined sugar from sugar beet, food grade, Südzücker AG, Mannheim, Germany) prepared with deionized water. The mixture was left for 10 days in an airtight vessel. After 10 days, the pH of the suspensions dropped from initially 10.1 to 4.5 and the fermentation was stopped by separating the biochar by removal of the biomass and draining the water. The lactic fermentation of pristine BC was included as an experimental treatment, as a modification of the initial BC pH may influence the nutrient release behavior of the respective BBF produced.

2.3. Experiential Setup

We implemented a full factorial design comprising 12 treatments, resulting from the combinations of the factors "fertilizer type" (four level: BC only, mineral fertilizer–NPK, BBF, fermented BBF) and the factor "type of root-zone application" (three level: homogeneous topsoil, root-zone hotspot, root-zone layer). An overview of factor-levels is presented in Figure 1. Pots with a volume of 5 L were used (25.5 cm height, 19.5 cm upper-diameter). For topsoil application the respective fertilizer was applied onto the topsoil prior to seedling transplantation and was homogeneously incorporated to a maximal depth of 10 cm. Layered root-zone application was achieved by applying the fertilizer as a consistent layer at 15 cm soil depth. For hotspot root-zone application, the fertilizer was applied in a hole (approximately 7 cm in diameter) at a 10 cm depth right in the center of the pot, i.e., directly under the later transplanted seedling. For BC treatment, the equivalent of 16.25 g DM biochar was applied per pot, for mineral fertilizer, 1.27 mL WUXAL (Düsseldorf, Germany), diluted with 150 mL deionized water, was applied.

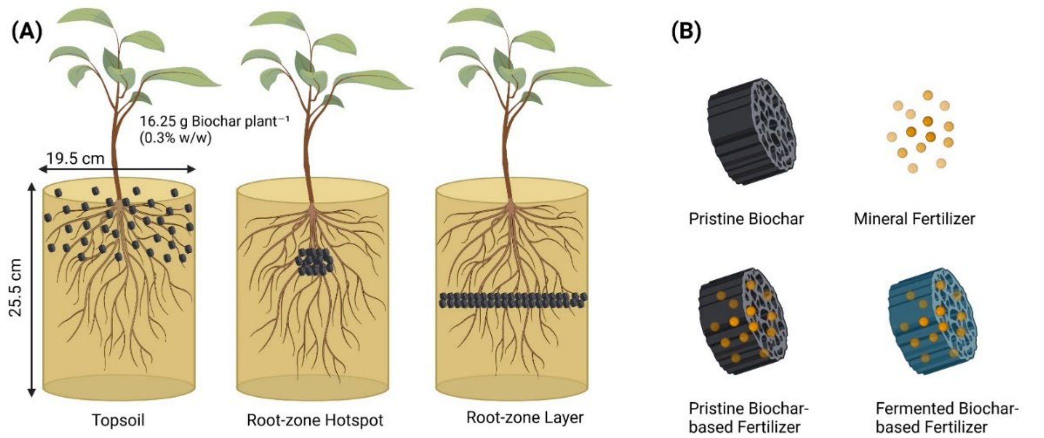

Figure 1. Visualization of the experimental treatments. (**A**): Fertilizer placement levels: Topsoil, root-zone hotspot and root-zone layer application. (**B**): Fertilizer levels: Pristine biochar (BC), mineral fertilizer (NPK), Pristine biochar-based fertilizer (BBF) and fermented biochar-based fertilizer (ferm. BBF). Each applied at rates of 16.25 g BC pot^{-1} (biochar micro-dosing), representing the equivalent of a 0.3% *w/w* amendment to the pot.

These 12 experimental treatments were complemented by a zero-control group and were set up in six replicates, which resulted in a total of 78 pots. The pots were arranged in six blocks in a randomized block design. The cocoa plants were allowed to grow for 153 days after the transplantation of seedlings. The experiment was set up in a semi-controlled climate chamber, programmed for 25 °C day-temperature and 20 °C night-temperature and a 12 h/12 h day and night rhythm; relative humidity was kept at an average of 50%. The soil was watered twice a week and re-adjusted to 60% water holding capacity, using deionized water.

2.4. Plant Analysis

Using an OPTI-Sciences CCM200-plus Spad-meter (Opti-Sciences Inc., Hudson, United States of America), the unitless Chlorophyll Content Index (CCI) was measured, further the total plant height and diameter was determined in bi-weekly intervals. The leaves of each plant were scanned in a LI-COR-3100-C leaf scanner (LI-COR Biosciences GmbH, Bad Homburg, Germany) and were analyzed for total plant leaf area (TLA). Using a wet-sieving procedure, the complete rootstock was extracted from the soil medium. Dry matter of both above ground biomass (ABG) and below ground biomass (BGB) were determined after drying at 60 °C for 48 h.

The dried leaves were milled using a RETSCH centrifugal mill (RETSCH GmbH, Haan, Germany). A 0.1 g subsample was then acid digested in 65% nitric acid (HNO_3) for 8 h at 185 °C in Teflon tubes. The digests were consecutively analyzed for P, K, sodium, sulphur, calcium, magnesium, manganese, iron and aluminum using inductively coupled plasma optical emission spectrometry (ICP-OES). A second subsample of 2–5 mg was analyzed in a CN-analyzer (Vario-Cube, Elementar, Langenselbold, Germany) for C and N content using dry combustion with coupled emission gas chromatography.

2.5. Statistical Analysis

Statistical analyses were carried out using the software R (Version 4.0.4) facilitated through the editor R-Studio. Multifactorial ANOVA models, explaining the dependent variable, using fertilizer-level, placement-level and block as predictors, and considering an interaction of fertilizer-level and placement-level were constructed. Each ANOVA-model was inspected for normality of residuals using a Shapiro-Wilk test and for variance homogeneity using residuals versus fitted diagnostic plots. The zero controls were excluded from this interaction model to guarantee for balanced designs. The preliminary models indicated a significant interaction of the factors fertilizer and placement but not of the factor block which could thus be removed from the model.

A second set of simplified one-way ANOVA models was constructed, considering the factor level combinations of the main effects (Treatments) as the only predictor. Here, the zero controls were reintroduced to the model to serve as a reference point, while keeping a balanced design. Again, these models were inspected for normality and homoscedasticity using Shapiro–Wilk and Levene tests. When ANOVA models indicated significant differences between treatment means, they were followed up by Tukey-HSD post hoc tests at alpha = 0.5 to identify and assign the respective homogeneous subgroups.

In the case of total aboveground biomass and foliar nitrogen, the condition of normally distributed model-residuals was not met and could not be established through box-cox-, logarithmic- or square root transformation. Therefore, these two models were analyzed by a Kruskal–Wallis test for nonparametric data, followed by the Dunn post hoc test with Bonferroni-correction. This test is considered very conservative, and the identification of significant differences is less likely (see boxplots chapter 3). All analyzed plant parameters were tested for correlations. As most variables did not follow the normal distribution, the Spearman rank correlation was used. Significant correlations were determined using a p-value < 0.05.

3. Results

3.1. Morphological and Physiological Characteristics

In the following, all relative changes are compared to topsoil applied mineral fertilizer (referred to as "farmer practice"), unless stated otherwise. After 22 weeks of cocoa growth in the greenhouse, the mean AGB ranged from 14.0 ± 0.7 g DM plant^{-1} (BC root-zone hotspot) to 26.5 ± 1.2 g DM plant^{-1} (BBF root-zone layer) while the Control showed 14.7 ± 0.9 g DM plant^{-1}. Root-zone layer application of BBF represented a 56% increase in AGB compared to farmer practice, whereas BBF root-zone hotspot application and fermented BBF in root-zone layer application increased yields by 51% and 46%, respectively (Figure 2A and Table S3). There is a strong tendency towards BBF having a greater, positive effect size on AGB than the application of either BC or NPK individually, the same as root-zone applications having a greater, positive effect size than topsoil applications; however, significant differences could not be identified (see Materials and Methods 2.4). All treatments without mineral fertilizer (BC and control) resulted in a lower AGB than the farmer practice (−11% to −18%). Primary data on plant height and diameter are presented in Figures S1 and S2.

The mean TLA ranged from 435 ± 127 cm^2 (Control) to 2485 ± 396 cm^2 (BBF root-zone layer). The latter was significantly different from all other treatments and showed an increase of 222% compared to farmer practice (Figure 2B and Table S4). All treatments without mineral fertilizer resulted in negative relative changes of up to 44% decrease in mean TLA compared to the farmer practice. Significantly higher TLA was achieved through all root-zone applications of NPK, BBF or fermented BBF, as compared to the farmer practice and to BC only amendments, regardless of their placement. Plants being subject to topsoil applications show little differences in mean TLA among the fertilizer treatments.

The mean Chlorophyll Content Index (CCI) ranged from 7.1 ± 0.9 (BC root-zone layer) to 19.8 ± 2.0 (BBF root-zone layer) and showed a pattern similar to that of TLA, yet the differences were found to be less pronounced (Figure 2C and Table S5). The control reached a mean CCI of 7.7 ± 1.3. The CCI resulting from BBF applied in a root-zone layer was significantly higher (+140% compared to the farmer practice) than any mineral fertilizer and BC treatments regardless of the placements.

The mean BGB was within the range of 6.4 ± 0.3 g DM plant^{-1} (BC root-zone hotspot) to 9.2 ± 0.9 g DM plant^{-1} (fermented BBF root-zone layer). The latter represents a BGB increase of 27% compared to the farmer practice (Figure 2D and Table S6). The control plants showed a mean BGB of 6.8 ± 0.2 g DM. Without mineral fertilizer addition, mean BGB was reduced by 6% (BC root-zone layer) and 11% (BC root-zone hotspot) compared to farmer practice. Root-zone hotspot and root-zone layer application of BBF resulted in a 21% and 16% increase of the mean BGB of cocoa seedlings, respectively. Mineral fertilizer application resulted in higher means of BGB when used in root-zone hotspot application compared to the two other placements.

In summary, BBF in the root-zone layer application resulted in higher mean values compared to the hotspot or topsoil application. However, both the root-zone hotspot and root-zone layer application resulted in greater variability among the replicates of the same treatment. The topsoil application of fertilizers resulted in less pronounced differences between different types of fertilizers, with generally inferior results.

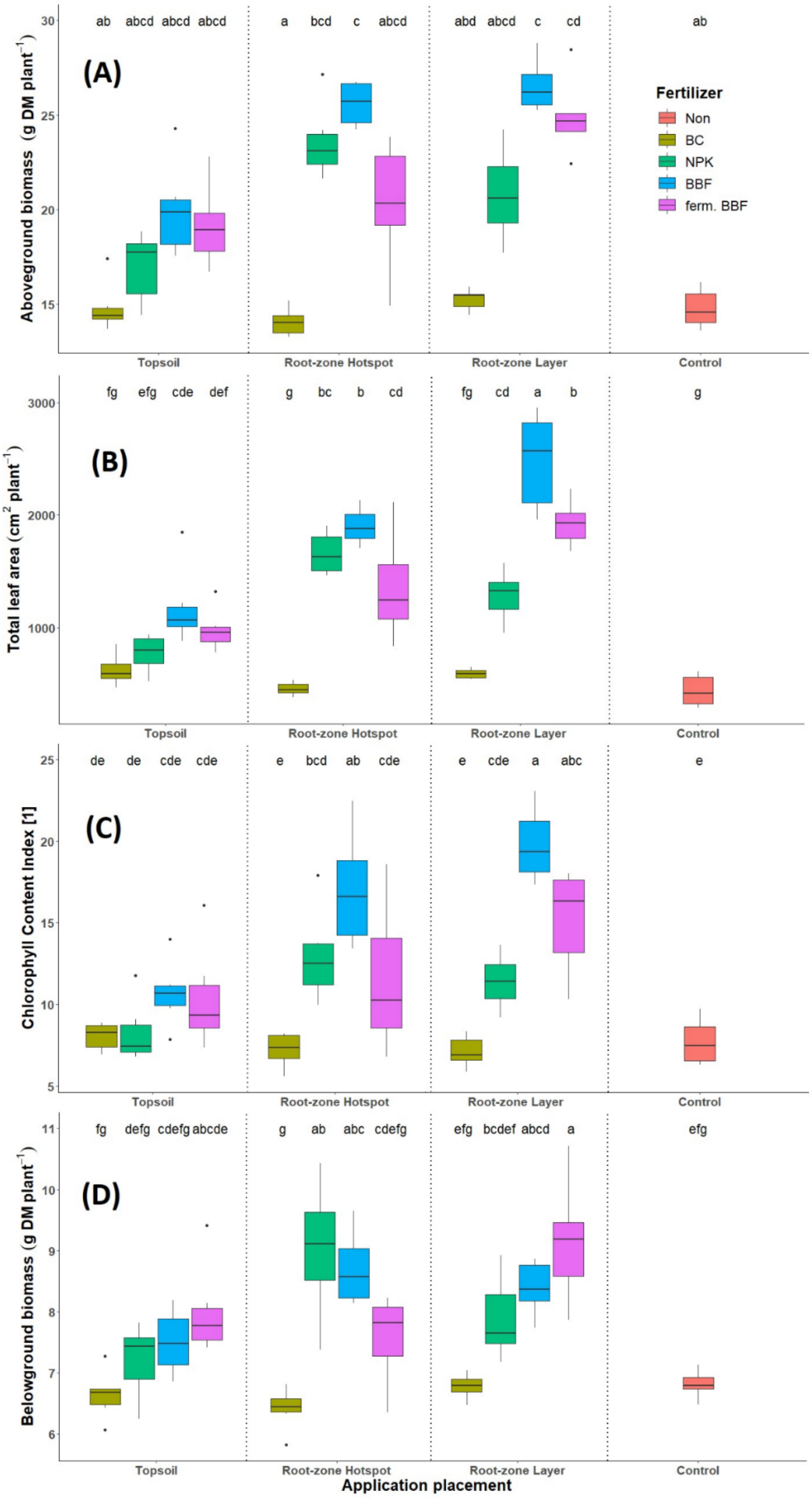

Figure 2. Boxplots of (**A**) aboveground biomass (g dry matter—DM), (**B**) total leaf area (cm^2 per plant), (**C**) Chlorophyll Content Index (unitless) and (**D**) below ground biomass (g DM) of cocoa plants after 153 days as a function of fertilizer type and type of root-zone application—with boxes indicating the 25% to 75% quantiles and the median as a line, whiskers are displayed as lines and outliers are displayed as dots (determined via 1.5*IQR rule). Treatments sharing a letter are not significantly different in means (Dunn test with Bonferroni correction in (**A**), Tukey-HSD-test for (**B**–**D**)).

3.2. Elemental Composition of Foliar Biomass

The P content in the foliar biomass of the BBF root-zone layer treatment was significantly higher than all NPK and BC treatments regardless of their placements. The mean P content of 0.97 ± 0.1 mg g^{-1} (0.097% of dry matter) was 53% higher than for the farmer practice. There was no significant difference between BBF root-zone layer, fermented BBF root-zone layer (0.84 ± 0.1 mg g^{-1}), and BBF root-zone hotspot (0.82 ± 0.1 mg g^{-1}, Figure 3A and Table S7). Except for the fermented BBF root-zone layer, the mean N:P ratio was above 16 for all treatments (Figure 3B). Data on further nutrients are available in the SI (N, K, Ca, Mg, Mn, S, Fe, Na cf. Figures S3–S5). Aluminum content was below the detection limit (<0.01 mg Al gDM^{-1}) for all treatments.

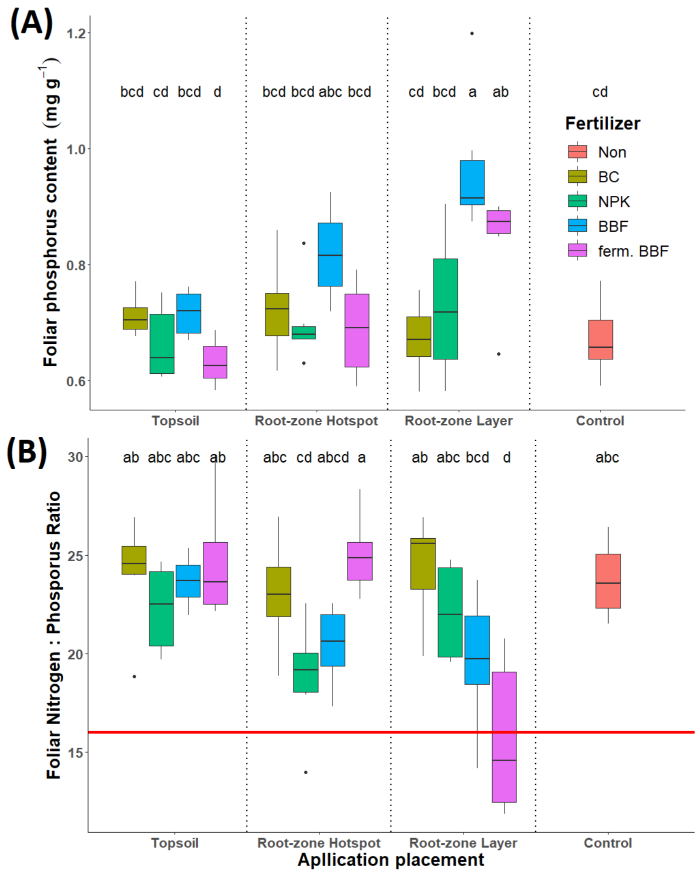

Figure 3. Nutrient content cocoa leaves after 153 days as a function of fertilizer type and type of root-zone application. (**A**) Foliar P content in boxplot with boxes indicating the 25% to 75% quantiles and the median as a line, whiskers are displayed as lines and outliers are displayed as dots (determined via 1.5*IQR rule). Treatments sharing a letter are not significantly different in means (Tuckey-HSD-test). (**B**) Nitrogen-to-phosphorus ratios of foliar biomass. The horizontal red line is indicating the N:P < 16 threshold at which P limitations are overcome.

Spearman rank correlation showed that morphological plant parameters seem to be highly correlated whereas the nutrient concentration in foliar biomass showed a less pronounced correlation (Figure 4). Besides P, calcium is also positively correlated with morphological plant parameters whereas increasing K contents fostered lower values for morphological plant parameters in this study. Primary data on plant height and diameter are presented in Figures S1 and S2.

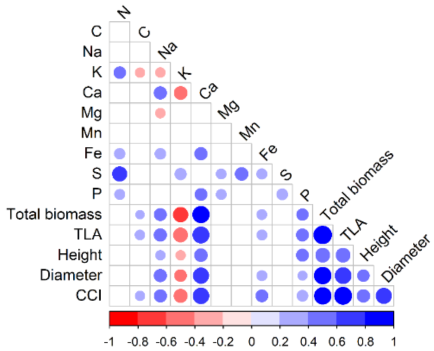

Figure 4. Spearman's rank correlation coefficients between foliar nutrient content and parameters of plant morphology and physiology with positive correlations in blue, negative in red and increasing circles size with increasing correlation. Empty boxes indicate non-significant correlations.

4. Discussion

Our greenhouse experiment revealed a significant increase of leaf phosphorous content, TLA, CCI and BGB by concentrated root-zone application of BBF prepared from mixing biochar with liquid NPK mineral fertilizer when compared to the farmer practice of topsoil NPK mineral fertilization. The root-zone layer application of BBF also showed a significant increase in leaf phosphorous content, total leaf area and CCI when compared with the best treatment without biochar (root-zone applications of NPK). Notable is the finding of BBFs capability to stimulate leaf area increases of up to + 222% compared to the farmer practice. *T. cacao*, being a shade bearing plant, has inherently low rates of photosynthetic activity [37,38]. An increase in leaf area can increase net assimilation and yield and is therefore considered a beneficial trait. The results of improved performance of mineral fertilizer charged BC aligns with the findings of Quaye and colleagues who deployed a BBF from rice-husk biochar and compost as the *T. cacao* growing medium [39]. They found a significantly higher leaf area of cocoa seedlings in BBF growth medium compared to the topsoil + compost medium, as well as increased height, shoot and root biomass and foliar P content too. Our results suggest that significant effects were achieved by combining BBF (instead of the application of pure biochar or mineral fertilizer) with concentrated root-zone application (instead of homogeneous application/ spreading). It is remarkable that these results were achieved through the micro-dosing of biochar, with an application rate equivalent to just 18 kg ha^{-1} biochar DM (16.25 g per plant, 1111 plants ha^{-1}.; i.e., 3 m × 3 m planting grid). When assuming a BC application mode proportional to a traditional fertilization pattern of a 1 m radius around the plants, 2 t BC ha^{-1} would be required. Such rates are in sharp contrast to many previous studies with BC application rates of 10–100 t ha^{-1} BC in pots [40] or 5–20 t ha^{-1} in the field [41]. This omnipresence of high application rates also mirrors thresholds of $\leq$5 t ha^{-1}, 5–10 t ha^{-1} or 10–20 t ha^{-1} as selected for meta-analysis comparing the effects of biochar and BBF applications [17].

4.1. Interaction of Biochar and BBF with Soil Nutrients

Most tropical agro-ecosystems are limited by N and P, whereby P is especially limiting plant growth in most Oxisols. This also holds true for tropical tree-based systems [42]. The application of pure BC can mobilize soil P in tropical soils that was not plant available prior to BC application due to an unfavorably low pH and the resulting immobilization of P as aluminum phosphate. A meta-analysis of 124 studies [18] concluded that BC application can increase soil P availability by 45%. In a subset of this meta-analysis, assessing only studies deploying the Olson method (alkaline extraction method for tropical soils), an increase of over 60% was identified. The authors suggest three major BC mechanisms: 1. altering the soil pH; 2. altering the P adsorption equilibrium; and 3. microbially mediated mechanisms, i.e., BC induced enhancement of microbial and mycorrhizal P mobilization by enzymes or chelates. All these processes were demonstrated for pure BC, but rarely for BBF. However, there is basically no indication that the application of BBF should not also lead to these effects. Still, since BBFs are usually also a carrier of (at least initially) plant-available phosphorus, as in our experiment, it is difficult to distinguish fertilizer-P and soil -P uptake unless isotopically labeled fertilizers are used. The use of isotopic labels was beyond the scope of our study.

Passive mobilization of P through BC-only additions is mainly relevant in slightly acidic soils with neglectable effects in strongly acidic soils [13]. Looking at our experiment, the application of pure biochar neither increased ABG, BGB nor foliar P content, compared to the zero control. Thus, potential biochar-induced mobilization of inherent soil P did not overcome nutrient limitations, at least in our short-term setup. However, the active addition of nutrients through mineral fertilizer did improve plant growth, despite the likely event of partial immobilization of added P through aluminum.

Depending on ambient soil conditions, the application of pure BC can result in undesired effects through the adsorption of nutrients which may lead to a decrease in yield (also observed in temperate soils) [43]. This may explain why the plants with pure BC root-zone hotspot treatment show a tendency to accumulate slightly less AGB than the control plants (Figure 2A). Elias and colleagues showed that the application of pure BC may be sufficient to partly ameliorate nutrient limitations in slightly acidic soils (pH 6.14), yet with respect to improved soil P availability, BC only treatments (30 t ha^{-1}) were inferior to fertilized controls. The increase in available soil P, as determined via PRS ion exchange membranes in a 1 day burial period, was greatest when facilitated through jointly applied BC and fertilizer (BBF); remarkably, in this joint application a negative correlation of soil P availability to the initial soil pH (higher soil P availability in soils with lower pH) was observed [13]. Another study by Aggangan and colleagues found that a BC application of 15% in a pot trial (*w/w*; as of 2000 g soil) to a soil with pH 4.1 significantly increases extractable soil P and the consecutive growth of *T. cacao* seedlings. Herein, interactions between BC and *Arbuscular mycorrhiza* (AM) further increased the effect size compared to BC or AM only [30]. It has to be noted that our application of 16.25 g biochar $plant^{-1}$ equals 0.32% (*w/w*; as of 5000 g soil) according to the calculation of Aggangan and colleagues. Sasmita and colleagues, who applied BC, with or without fertilizer, to the growing media (pH 3.9) of T. cocoa seedlings concluded that increased BC rate and fertilizers application elevated the available P and other exchangeable cations (K, Ca, Na), while reducing the exchangeable Al of the medium [29]. In essence, pure BC amendments to acidic soils can be beneficial. However, using BBF may allow the achievement of positive impacts with considerably lower and thus more economically viable biochar application rates. Both in less and strongly acidic soils, it might be recommendable to combine the BC with nutrients prior to application, by soaking biochar in mineral fertilizer solution. Previous studies [17,32] showed that BBF based on the combination of BC and mineral fertilizer resulted in better plant performances than individual applications of one of the two. The main reason is that the porous BC matrix was saturated with nutrients prior to soil application, which in consequence minimizes the post-application immobilization of other nutrients from the soil solution [17].

Despite all the positive impact of mineral fertilizers, BC and BBFs, all means in foliar P contents of all treatments, including zero-control, were well below the critical limit of 0.2% [44]. This supports the obtained N:P ratios of predominantly >16, indicating phosphor as the major limitation for the cocoa plants [20,34]. The application of BBF could significantly increase the P content compared to pure BC or mineral fertilization. Nevertheless, for this study all plants showed deficits in P as implied by the foliar P contents < 0.2% and N:P ratios > 16. This can be explained through neglectable initially available P in the soil (see soil analysis in Table S1) and immobilized P through aluminum-P complexes, also in the case of BBFs.

4.2. *Improved Effects of BBF by Concentrated Root-Zone Application*

The root structure of cocoa includes one major tap root and a net of lateral roots that grow in the first 20 cm of the topsoil [44]. These lateral roots are acquiring most of the needed water and nutrients. Soil amendments and fertilizers in our study, i.e., mineral fertilizer, BC and two BBFs, overall had a greater impact on plant growth when applied to the root-zone instead of the usual topsoil application. However, for soluble mineral fertilizer no difference was expected for different placements. The superior performance of the root-zone hotspot placement could be explained through the limited availability of native soil nutrients and the benefit for the cocoa plant if the fertilizer is readily available below the root mass at initial planting. Over time this benefit may vanish as the soluble nutrients distribute evenly through the soil according to concentration gradients and mobile nutrient loads are absorbed by the plant. Our results are in good agreement with recent field trial investigations on functional traits of roots in relation to the fertilization of cocoa agroforestry systems. The latter study established fertilization plots (1 m $\times$ 2 m; n = 27) in which NPK 15:15:15 fertilizer was broadcasted at two different rates. In-growth cores were used to extract roots from two soil depths. [45]. They found that, in surface soils (0 to 10 cm, comparable to depth of topsoil application in this study), fertilization largely stimulated roots to express resource conservative strategies (less root biomass) compared to unfertilized cocoa roots. This could explain the limited effect sizes of topsoil application with different fertilizers for all investigated cocoa plant parameters, including BGB, as compared to deeper fertilizer placements. Furthermore, their data on root-traits in subsurface soils (10 to 20 cm, comparable to depth of root-zone layer application in this study) implied an increasing complexity in nutrient acquisition strategies with soil depth. Similarly, biochar has the potential to improve nutrient availabilities in multiple complex ways [9,18], thus potentially more positive feedbacks are promoted in the subsoil. This array remains a subject for further research.

When BBF is applied, the associated mineral fertilizer reaches the rhizosphere in a place that is physically and chemically altered by the jointly applied BC. This alteration, including colloidal structures, pH and soil microbiome [18], provides an environment that improves the plant availability of the applied fertilizer—particularly P—while also bearing the potential of mobilizing soil P, thus increasing its total uptake potential, as mirrored in the elevated foliar P contents. The tendency to perform better in the root-zone layer than in the root-zone hotspot application could be explained by an increase in surface area for roots to take advantage of stored nutrients and a larger volume of the rhizosphere being subject to alteration.

Regarding BGB, significant differences between the application of BBF and either BC or NPK applications can be observed, whereby fermented BBF showed the best performance in both topsoil and root-zone applications. Overall, the magnitude of absolute differences in BGB, even though significant, are small. This is likely due to the prevalence of free aluminum. Aluminum is capable of permanently replacing calcium from the root-cell membranes and consecutively inhibiting cell elongation and growth [46]. The rather concentrated application of BC/BBF, especially in the root-zone, leaves most of the soil volume unaltered, providing the same unfavorable growing conditions for roots, across all treatments. Application to the root zone allows for spatially limited, improved nutrient uptake by the roots, but little improvement of overall growth in the given soil volume. Thus, when cocoa is planted in the field, root-zone layer application could be a cost-effective tool to boost the growth of the seedling; however, it will require repeated applications as the plant grows. This could be done by digging up to 20 cm deep trenches to be partially filled with BBF (vertical mulching), as demonstrated for tea [47] or by novel geo-injection technology, such as, for example, VOGT Geo-tec (Weidenberg, Germany) [48]. Despite additional work, the timely application of BBF in the root zone of cocoa might improve plant growth and yield the reasonable amount of biochar needed. This approach would also be easier to reconcile with on-site production of biochar (continuous production, but of rather small quantities) and with the Kon-Tiki flame-curtain pyrolysis [49,50], since this mode of production in the tropics may be more economically viable for a cocoa farmer than the one-time purchase of a large quantity of biochar from an industrial pyrolysis plant. An assessment of smallholder cocoa agroforestry systems in Ghana concluded that

2.6 t ha^{-1} year^{-1} of underutilized biomass (prunings and pods) are available for biochar production [51]; other studies point towards even higher quantities [52].

5. Conclusions

The application of BBF can improve the performance of *T. cocoa* plant growth and vigor beyond the levels achieved with either pure mineral fertilization or pure BC. The high potential of biochar micro-dosing as part of an optimized nutrient management and the strong influence of the BBF placement is remarkable. A layered root-zone application promoted the most beneficial BBF × rhizosphere × plant interaction, especially regarding P acquisition. The increased vigor (AGB, BGB, TLA, CCI) is expected to result in improved survival rates and potentially increased future crop yields.

Upscaling and mainstreaming the production and application of biochar-based fertilization in tropical agroecosystems can substantially improve the on-farm nutrient management while building up stable carbon-sinks; thus bearing the potential to support several Sustainable Development Goals from SDG2 (zero hunger) to SDG13 (climate action).

Still, data from the greenhouse need to be validated in the field. In particular, this includes the test of different types of root-zone application. The practice must show whether the layer application can prove itself and how it can be implemented and optimized. When moving from greenhouse to field trials, focus must be placed not only on the underlying mechanisms, but also the practicability of integrated on-farm biochar production and root-zone application technologies (e.g., geo-injection). Basic and applied research targeting the integrated production and application of BBF in tropical (perennial-) agriculture is at the heart of the global climate–water–food nexus research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8040328/s1, Table S1: Soil characterization; Table S2: Biochar characterization; Table S3: Mean above ground biomass and relative increases; Table S4: Mean leaf area and relative increases; Table S5: Mean Chlorophyll Index and relative increases; Table S6: Mean belowground biomass and relative increases; Table S7: Mean foliar phosphorous content and relative increases; Figure S1: Plant height growth curve; Figure S2: Diameter at base development curve; Figure S3: Foliar Nitrogen content; Figure S4: Foliar Potassium content; Figure S5: Foliar Calcium, Sulfur, Sodium, Manganese, Iron and Magnesium contents.

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