# Influence of co-applying biochar, compost and inorganic nitrogen on growth, nutrient uptake and nitrates and riboflavin content of turnips

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**Abstract**

**In the pursuit of sustainable vegetable farming methods, the effect of co-application of biochar, compost, and a reduced amount of inorganic nitrogen on growth and the quality of turnips was studied in a greenhouse pot experiment. The experiment was carried out with six fertilizer treatments: unfertilized soil (US), compost + biochar (C+B), compost + 0.5 g N/pot (C+0.5N), biochar + 0.5 g N/pot (C+0.5N), compost + biochar + 0.5 g N/pot (C+B+0.5N), and pre-plant mineral fertilizer + 1 g N/pot (PF+N). Municipal organic compost collected selectively (150 g/pot), biochar (20 g/pot), and pre-plant mineral fertilization were mixed with the top 10 cm of soil. Inorganic nitrogen, was applied weekly in equal amounts. All treatments increased the N, P and K uptake and dry weight of roots and shoots, compared to unfertilized soil. Plant dry weight increase in the treatments C+0.5N, PF+N, and C+B+0.5N relative to the unfertilized soil was 767.7%, 734.9%, and 687.4%. Adding biochar to C+0.5N reduced Ca and Mg plant (root and shoots) uptake but unaffected the biomass accumulation. The root, shoot, and total plant dry of plants grown with C+0.5N and C+B+0.5N was not significantly different from those grown only the inorganic fertilization (PF+N). Turnips grown with C+0.5N and C+B+0.5N accumulated similar biomass to those with inorganic fertilization (PF+N), while also reducing nitrate content. The co-application of compost with a reduced amount of nitrogen, can potentially eliminate or reduce the need for inorganic N, K, P, Ca and Mg while maintaining yield and quality. This study indicates that the addition of biochar to compost and inorganic nitrogen unaffected biomass accumulation, but reduced shoot riboflavin and thiamine contents of turnips.**

**Keywords:** *Brassica rapa L., biochar, municipal compost, carbon sequestration, circular economy, shoot nitrate, sustainable vegetal farming.*

## INTRODUCTION

Adding biochar to soil is seen as a promising way to improve fertility and plant growth while boosting carbon sequestration and potentially reducing nitrate leaching and nitrous oxide emissions (Borchard et al., 2019; Siedt et al., 2022; Mousavi et al., 2023). However, its effect on plant growth can vary with soil type, biochar quality, application rate, etc. The application of biochar may improve, or at least not harm, plant productivity (Biederman and Harpole, 2013) or negatively affect plant productivity (Kloss et al., 2014; Calderón et al., 2015). Biochar addition to soil alone has limited impacts on increasing yields (Li et al., 2019).

For biochar to have positive effects, it is necessary to combine its application with inorganic fertilizers (Bai et al., 2022), particularly N (Saha et al., 2019) or compost (Qian et al., 2023). Biochar may decrease soil N availability due to N immobilization (Ippolito et al., 2012; Calderón et al., 2015), ammonium retention, and physical entrapment of nitrate (Gelardi et al., 2021), potentially limiting crop growth in the short term. Combining N fertilizer with biochar improved N fertilizer use and maize production by enhancing soil fertility, crop uptake, and plant growth (Li et al., 2023). Combining biochar with compost application has been reported as a promising strategy to promote plant growth (Liu et al., 2021; Qian et al., 2023). The application of biochar plus compost (Chen et al., 2022) along with inorganic fertilization increased plant productivity (Naeem et al., 2018). However, the effect of the mix of biochar with organic materials on plant growth was mainly dependent on the chemical quality of the of the organic materials. The synergistic effects were prevalent when nitrogen-rich and lignin-poor materials were mixed with biochar (Bonanomi et al., 2017). According to Amlinger et al. (2004), only about 5% to 15% of the total N supplied by mature compost is available in the first year after application. Therefore, the contribution of compost to soil N availability is relatively low. Consequently, the application of biochar alongside compost could also benefit from the addition of some inorganic N. Furthermore, the combined application of inorganic N with organic compost may contribute to reduction inorganic N (Machado et al., 2020) and another inorganic nutrient without reduce yield (Machado et al., 2022). Thus, the main goal of this research is to analyze the influence of co-applying biochar, compost, and a reduced amount of inorganic N on plant growth, nutrient uptake, and root and shoot nitrate, riboflavin, and thiamin concentrations, as well as peroxidase enzyme activity in turnips grown in low-fertility soil.

## MATERIALS AND METHODS

### Growth conditions and substrates

The study was conducted in a greenhouse located at the "Herdade Experimental da Mitra" (38°57′ N, 8°32′ W), University of Évora, Portugal. The greenhouse was covered with polycarbonate and had no supplemental lighting. Air temperatures inside the greenhouse ranged from 3 to 26 °C and outside solar radiation ranged from 31.3 to 226,9 $W{\cdot}m^{-2}{\cdot}d^{-1}$.

The experiment was carried out in plastic pots. Each 12 L plastic pot (21 cm high × 27 cm diameter) was filled with 14 kg of loamy sandy soil obtained from the upper 30 cm soil of the Mitra Research Farm. Portugal. The soil presented a 1.2% organic matter content, a bulk density of 1.47 $g{\cdot}cm^{-1}$, and a pH of 5.7 (1: 2.5 soil-to-water distilled water ratio, w/v), an electrical conductivity (ECe) of a saturated paste extract of 0.3 d*S* $m^{-1}$, 75 mg $K{\cdot}kg^{-1}$, 79 mg $P{\cdot}kg^{-1}$, 1.16 meq $Ca^{2+}$/100 g, and 0.57 meq $Mg^{2+}$/100g.

The experiment was carried out with six fertilization treatments, which involved the application of compost (150 g /pot), biochar (20 g/pot), inorganic fertilizers applied in pre-plantation and inorganic N applied weekly. The characteristics of compost and biochar, along with their raw material origins, are detailed in Machado et al. (2021) and Machado et al. (2022). The treatments were as follows: unfertilized soil (US), compost + biochar (C+B). compost + 0.5 g N/pot (C+0.5N), biochar + 0.5 g N/pot (B+0.5N), compost + biochar + 0.5 g N/pot (C+B+0.5N), and pre-plant mineral fertilizer + 1 g inorganic N/pot (PF+ N).

Fifteen days prior to transplanting, mature municipal solid waste organic compost in pulverulent form, biochar, and inorganic fertilizers applied in pre-plantation were added to each pot of respective treatment and incorporated into the top 10 cm of soil. In the pre-plant mineral fertilization was applied 0.17 g N, 0.36 g $P_2O_5$, 0.59 $K_2O$, 0.33 CaO, and 0.13 g MgO. N was applied as ammonium nitrate (16.9% $NO_3$–N and 16.7% $NH_4$–N) applied via fertigation once a week in equal fertilizer applications. starting at transplantation and finishing two weeks before harvest. Treatments were arranged in a randomized complete block design with five replicate pots per treatment.

Turnip seedlings (*Brassica rapa* L. cv. Falko) were transplanted on January 7, 2023, into 12 L pots. Plant irrigation was based on volumetric soil water content measurements taken daily (8:00–9:00 h) with a soil-moisture probe (SM105T Delta devices UK). When the average soil moisture in the top 0.1 m, measured at 7cm from the center of the pots, of treatment PF + 1N was ≤ 20%, plants were watered by hand (9:00–10:00), avoiding applying high volumes of water to minimize drainage losses. The weeds were regularly removed from the pots manually.

## Measurements

The plants were harvested 62 days after transplantation and separated into roots and shoots. Four plant shoots and roots from each treatment were washed and oven-dried at 70 °C for 2-3 days, weighed, and ground so that they would pass through a 40-mesh sieve. then analyzed for N, P, K, Ca, Mg, and nitrates. N was analyzed by using a combustion analyzer (Leco Corp., St. Josef, MI., USA). The K was analyzed by flame photometry (Jenway, Dunmow, UK). The P was analyzed using a UV/Vis spectrometer (Perkin Elmer lamba25). The Ca and Mg were analyzed using an atomic absorption spectrometer (Perkin Elmer, Inc., Shelton, CT, USA). The contents of chlorophyll a (Ch a), chlorophyll b (Ch b), and carotenoids (Cc) of fresh weight were determined using the methodology described by Machado et al. (2023). Nitrates were determined in accordance with Lastra (2003). Samples of 1.0000 g of *Brassica rapa* leaves and roots, macerated in liquid $N_2$ were homogenized in 0.12 mM phosphate buffer and PVP, pH 7.2, at 4 °C. The homogenate was centrifuged at 12,000 *g* for 20 min. Aliquots of the supernatant (buffered extract, BE) were used to quantify riboflavin, thiamine, and peroxidase enzyme activity (POD), in accordance with Nisha et al. (2005), Plaza (2003), and Hernández and Cano (1998), respectively.

## Data Analysis

Data were processed via analysis of variance using SPSS Statistics 25 software (Chicago, IL, USA). licensed to the University of Évora. Means were separated at the 5% level using Duncan's new multiple range test.

# RESULTS AND DISCUSSION

## Shoot and root macronutrient uptake

All treatments increased the uptake of N, P, K, Ca, and Mg by roots and shoots compared to unfertilized soil (US), except for root Ca and Mg uptake in the C+B treatment (Table 1). Macronutrient uptake by roots and shoots grown with C+0.5N was significantly higher than that grown with B+0.5N. Adding biochar to the C+0.5N treatment did not affect the uptake of N, P, and K by the shoots or the P uptake by the roots compared to using C+0.5N alone. However, it significantly increased the uptake of N, K, and Ca by the roots (Table 1) and decreased the uptake of Ca and Mg by the shoots as well as Mg uptake by the roots (Table 1).

Plants grown exclusively with inorganic fertilization (PF+N) took up significantly more N (760.7 mg per shoot and 527.7 mg per root) than those grown in the C+0.5N and C+B+0.5N treatments (Table 1). However, K uptake in these treatments increased by an average of 87% in the roots and 120% in the shoots, compared to plants grown with inorganic fertilization alone. The addition of biochar to compost and 0.5 N increased root K uptake relative to all other treatments. This increase can be related to both compost and biochar application. Biochar increased K availability in soil (Wang et al., 2018; Bornø et al., 2019) and plant tissue concentration (Bierderman and Harpole, 2013). K release from organic matter is primarily driven by water and is not significantly limited by organic matter decomposition (Brito et al., 2014; Andrews et al., 2021). Unlike N and P, K is not incorporated into organic matter (Andrews et al., 2021), However, compared to plants grown only with inorganic fertilizers, the combined application of compost and 0.5N increased P uptake by the roots (Table 1) and by the plant. The combination of organic and mineral fertilizers, may improve P solubilization, reduce P precipitation, and, thus, increase the P availability for crops (Meena et al., 2018). Compost can improve P availability not only by directly supplying P but also indirectly by adding humic substances, alterations in soil pH, and an increase in diffusive efflux (Machado et al., 2021). This indicates that the co-application of compost and a reduced amount of N, with or without biochar, may reduce or even eliminate the need for inorganic K, and P. Machado et al. (2021) also reported that the combined application of compost with N can replace inorganic P and K fertilization.

Plants grown with C+0.5 N uptake more Ca (227.8 mg/plant) than those grown with C+B+0.5N (159.6 mg/plant) and those with inorganic fertilization (PF + N) (189.1 mg/plant). Plant Mg uptake in plants grown with C+0.5 N (86.4 mg/plant) was higher than that in those grown with C+B+0.5 N (46.1 mg/plant) and similar to that in those grown with inorganic fertilization (83.6 mg/plant). Thus, the co-application of C+ 0.5 N can also potentially reduce or eliminate Ca and Mg fertilization. To better understand how treatments influence plant nutrition, future research should examine their impact on soil nutrient availability.

Table 1- Effect of fertilizer treatments on N, P, K, Ca and Mg uptake by turnip shoots and roots.

| | Shoot macronutrients (mg/shoot) | | | | | Root macronutrients (mg/root) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | N | P | K | Ca | Mg | N | P | K | Ca | Mg |
| US | 54.67 e[1] | 12.1 e | 44.1 f | 32.8 e | 5.5 e | 28.6 e | 20.7 e | 35.9 f | 10.5 d | 4.5 d |
| B + 0.5 N | 412.37 c | 52.3 c | 116.7 e | 137.3 b | 33.4 b | 294.7 d | 93.0 c | 165.0 d | 26.2 c | 36.7 b |
| C + 0.5 N | 532.33 b | 91.9 b | 505.1 a | 203.3 a | 32.5 b | 448.5 c | 223.0 a | 519.6 b | 24.5 c | 53.9 a |
| C + B | 125.80 d | 29.2 d | 166.2 e | 69.6 d | 17.2 d | 70.9 d | 58.4 d | 129.3 e | 10.1 d | 6.6 d |
| C+ B+ 0.5N | 552.38 b | 96.4 b | 459.8 a | 107.5 c | 28.7 c | 475.7 b | 197.7 a,b | 564.8 a | 52.1 a | 17.4 c |
| PF+ N | 760.73 a | 108.8 a | 226.9 b | 157.5 b | 55.0 a | 527.4 a | 182.6 b | 288.5 c | 31.6 b | 28.6 b |
| Significance | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |

[1] - Means followed by different letters within a column are significantly different at $P \leq 0.05$. *** significant at $p < 0.001$ levels. respectively (US- unfertilized soil. C- compost. B- Biochar. N – nitrogen, PF – pre-plant fertilization)

## Plant growth and yield

All treatments increased the fresh and dry weights of roots and shoots compared to unfertilized soil (US) (Table 2). The highest increases in the treatments where amendments were applied were observed in plants grown with C+0.5N and C+B+0.5N. The addition of biochar to C+0.5 N did not significantly affect the fresh or dry weight of roots or shoots compared to plants grown with C+0.5 N (Table 2). Plants grown only with inorganic fertilization had higher shoot and root fresh weight than those grown with C+0.5 N and C+B+0.5 N (Table 2). However, despite the differences in nutrient uptake (Table 2), the root, shoot, and plant dry weights of the plants grown with C+0.5 N and C+B+0.5 N were not significantly different from those grown with inorganic fertilization (PF+N), where a double amount of N was applied (Table 2). Plant dry weight increase in the treatments C+0.5 N, PF+N, and C+B+0.5 N relative to the unfertilized soil was 767.7%, 734.9%, and 687.4%, respectively. This indicates that using compost along with a reduced amount of N permits reducing the application of inorganic fertilization and maintaining dry matter production levels similar to those achieved with inorganic fertilization. Although adding biochar to C+0.5 N did not increase dry weight accumulation, it contributes to carbon sequestration in the soil and can be advantageous in the long term.

Table 2 - Effects of fertilizer treatments on root and shoot dry weight and fresh weight of turnip.

| | Fresh weight (g/plant) | | | Dry weight (g/plant) | | |
|---|---|---|---|---|---|---|
| Treatments | Shoot | Root | Plant | Shoot | Root | Plant |
| US | 23.2 e[1] | 43.6 e | 66.8 e | 1.6 e | 2.6 e | 4.2 e |
| B + 0.5 N | 102.8 c | 146.0 c | 248.8 c | 8.2 c | 13.1 c | 21.3 c |
| C + 0.5 N | 160.8 b | 241.6 b | 402.4 b | 12.1 a | 24.5 a | 36.6 a |
| C + B | 49.2 d | 111.2d | 160.4 d | 3.6 d | 6.0 d | 9.6 d |
| C + B + 0.5 N | 165.4 b | 252.4 b | 417.8 b | 11.5 ab | 21.7 ab | 33.2 ab |
| PF + N | 172.4 a | 270.4 a | 442.8 a | 12.7 a | 22.5 ab | 35.2 a |
| *Significance* | *** | *** | *** | *** | *** | *** |

[1] - Means followed by different letters within a column are significantly different at P ≤ 0.05. *** significant at p < 0.001 levels. respectively (US- unfertilized soil. C- compost. B- Biochar. N – nitrogen, PF – pre-plant fertilization)

## Photosynthetic pigment

Fertilizer treatments significantly affected shoot photosynthetic pigment content (Table 3). Shoot chl a and b content in plants grown with B+0.5 N was lower than in those grown with C+0.5 N, C+B+0.5 N, or only with inorganic fertilization. Conversely, the plants grown with B+0.5 N had the highest shoot concentration (5 mg/100 FW) in Cc (Table 3). In the treatments (C+0.5 N, C+B+0.5 N, and PF+N), shoot Chla and Cc content were not significantly different. Thus, the addition of biochar to compost plus 0.5 N did not significantly affect the shoot Chla and Cc concentration.

Table 3 - Effect of fertilization treatments on shoot and root photosynthetic pigments of turnip.

| | Cha | | Chb | | Cc | |
|---|---|---|---|---|---|---|
| | mg/100g FW | | | | | |
| Treatments | Shoot | Root | Shoot | Root | Shoot | Root |
| US | 23.95 a[1] | 1.18 a | 21.27 a | 3.04 a | 1.02 c | Nd [2] |
| B+ 0.5 N | 3.03 c | 0.49 b | 2.54 d | 1.29 b | 5.08 a | Nd |
| C+ 0.5 N | 18.71 a,b | 0.42 b | 8.52 b,c | 1.08 b | 3.94 b | Nd |
| C + B | 18.42 a,b | 0.54 b | 6.03 c | 1.43 b | 3.77 b | Nd |
| C+ B+ 0.5N | 16.86 b | 0.44 b | 9.10 b | 1.06 b | 4.06 b | Nd |
| PF+N | 19.42 a,b | 0.45 b | 9.39 b | 1.23 b | 3.64 b | Nd |
| Significance | *** | *** | *** | *** | *** | |

[1]-Means followed by different letters within a column are significantly different at P ≤ 0.05. *** significant at p < 0.001 levels. [2]- not detected, (US- unfertilized soil. C- compost. B- Biochar. N – nitrogen, PF – pre-plant fertilization)

## Nitrates, riboflavin, thiamine and peroxidases

Plants grown with amendments and 0.5 N had higher root and shoot nitrates concentrations than those grown without inorganic N (US and C+B) (Table 4). Despite the increase, the nitrates concentration in roots and shoots was low, ranging from 0.08 to 0.27 mg/g FW. These values were lower than those considered usual for turnips (0.5 to 1 mg $NO_3^-$/g FW), which is a moderately nitrates-accumulating species (Santamaria, 2006). The addition of biochar to C+0.5 N did not affect nitrates content in shoots. Inorganic fertilization significantly increased nitrates content in both roots (0.66 mg/g FW) and shoots (1.02 mg/g FW) (Table 4). Leaf nitrates content increased linearly with the amount of N applied (Vieira et al.,1997). Nitrates concentrations in the roots and shoots were similar across different treatments, except for the PF+N treatment (Table 4). This contrasts with the results of Antonious et al. (2023), who reported a higher nitrates content in the roots than the shoots.

Plants grown in unfertilized soil (US) had the highest shoot riboflavin content (209.3 µg/100 g FW). The application of 0.5 N plus amendments led to a decrease in shoot riboflavin content compared to treatments without 0.5 N or those with only inorganic fertilization (Table 4). Biochar addition to C+0.5 N compared with C+0.5 N led to a decrease in shoot riboflavin content, but it did not affect root riboflavin content. Root riboflavin was lower in plants grown with inorganic fertilization and B+0.5 N. Riboflavin content was higher in the shoots than in the roots across all treatments (Table 4).

Plants grown unfertilized soil had the highest root thiamine content (15,8 µg/100 g FW) (Table 4). Root thiamine content in plants grown with amendments plus 0.5N and with inorganic fertilization was not significantly different. Plants grown only with inorganic fertilization (PF+N) had the lowest shoot thiamine content. Cartea et al. (2010) reported that riboflavin and thiamine are abundantly present in the top greens of the turnip. The results of the present study indicate that thiamine content in roots can exceed that in leaves. The thiamine values of turnip shoots and root ranged between 0.5 and 5.3 µg/100 g FW, and 7,1 and 15.8 µg/100 g FW, respectively (Table 4).

Fertilizer treatments had a significant effect on root POD activity but did not affect shoot POD activity (Table 4). The root POD activity was highest in turnips grown with PF+N C+0.5 while the lowest levels were found in plants grown with C+ B and C+B+0.5N. POD activity in the roots was higher across all treatments compared to the leaves (Table 4). POD plays a crucial role in scavenging toxic reactive oxygen species (ROS), modulating the antioxidant system, and enhancing photosynthetic efficiency (Sehrish et al., 2024). Plants grown with PF + N and C + 0.5 N showed higher root POD activity, potentially indicating greater stress tolerance. Adding biochar to C + 0.5 N, relative to C + 0.5 N alone, did not significantly affect peroxidase activity.

Table 4 - Effects of fertilizer treatments on root and shoot nitrates, riboflavin, thiamine and POD of turnip.

| | Nitrates | | Riboflavin | | Thiamine | | POD | |
|---|---|---|---|---|---|---|---|---|
| | mg/g FW | | µg/100g FW | | | | nmol.min$^{-1}$/mg | |
| **Treatments** | **Shoot** | **Root** | **Shoot** | **Root** | **Shoot** | **Root** | **Shoot** | **Root** |
| US | Nd | Nd [2] | 209.3 a | 72.6 a | 2.11 c | 15.83 a | 12,1 b | 348.4 a,b,c |
| B + 0.5 N | 0.26 b[1] | 0.27 b,c | 70.6 d,e | 13.2 c | 4.72 a,b | 9.72 b,c | 49.1 a | 330.2 b,c |
| C + 0.5 N | 0.22 b | 0.30 b | 83.8 d | 46.9 b | 5.26 a | 11.40 b | 17.7 b | 392.3 a,b,c |
| C + B | 0.08 c | 0.10 d | 125.5 c | 68.2 a | 3.88 b | 7.09 c,d | 45.3 a | 194.4 c |
| C + B + 0.5 N | 0.26 b | 0.24 c | 61.3 e,f | 43.4 b | 2.06 c | 11.79 b | 33.8 a,b | 249.9 c |
| PF + N | 1.02 a | 0.66 a | 145.8 b | 12.9 c | 0.47 d | 9.87 b,c | 19.1 b | 456.0 a |
| Significance | *** | **** | *** | *** | *** | *** | *** | *** |

[1]-Means followed by different letters within a column are significantly different at P ≤ 0.05. *** significant at p < 0.001 levels. Respectively. [2] Nd- not detected (US- unfertilized soil. C- compost. B- Biochar. N – nitrogen, PF – pre-plant fertilization)

## Conclusions

The addition of biochar to compost and inorganic N, compared to the co-application of compost and inorganic N, did not affect shoot N, P, and K uptake or the dry matter weight of roots and shoots. Plants grown with the co-application of compost and a reduced amount of inorganic N, with or without biochar, achieved root and shoot dry weights comparable to those obtained with inorganic fertilization. The co-application of compost with a reduced amount of inorganic N, allows for reduced or eliminated use of inorganic N, P, K, Ca, and Mg. Additionally, it reduces nitrates levels in the shoots and increases their thiamine content compared to using only inorganic fertilizer. These results indicate that using compost with reduced inorganic N, with or without biochar, can achieve high yields while boosting sustainability. However, further research is needed to assess how co-application affects soil nutrient availability to optimize fertilization strategies.

**ACKNOWLEDGEMENTS**

This work was funded in Portugal by the FCT - Foundation for Science and Technology - as part of Project UIDB/05183/2020.

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