

# *Microgranular biochar improves soil fertility and mycorrhization in crop systems*

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#### **RESEARCH ARTICLE**



## **Microgranular biochar improves soil fertility and mycorrhization in crop systems**

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#### **Abstract**

Intensive agricultural practices have accelerated soil organic carbon mineralization, compromising soil health and function. This study evaluated the efficacy of microgranular biochar (MicroCHAR) and powdered biochar as soil additives enhancing soil function, and pea, maize and wheat growth and yield. We carried out a series of experiments with degraded drought-prone soils in greenhouse and field conditions, combining biochar addition with arbuscular mycorrhizal fungi (AMF). The combination of amendments variously impacted soil nutrient status; availability of extractable potassium (K) increased in all cases, whilst that of calcium (Ca) was reduced when AMF inoculation was applied alone but not in combination with biochar. MicroCHAR positively affected root biomass and pea P content compared with the control, but biochar did not enhance N or K. Crop yield was not significantly increased by MicroCHAR amendment. MicroCHAR enhanced the mycorrhization rate of crop roots by 260%, an effect seen in the greenhouse and field conditions. This study suggests that credible benefits in some crops can be gained by the application of MicroCHAR to some soils. Observed effects may be soil and crop specific; future study of optimal nutrient and microorganism coatings on microgranular biochar opens exciting avenues for the improvement of crop yields in degraded agricultural soils.

#### **KEYWORDS**

biochar, crop yield, microorganisms, soil nutrients

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## **1** | **INTRODUCTION**

In pursuing high crop yields and global food security, the intensification of arable agriculture has triggered a rapid depletion of soil carbon (C) stocks (Stoate et al., [2001\)](#page-14-0). Over the decades, intensive cultivation practices, including frequent tillage, monocropping and synthetic fertilizer application, have accelerated the loss of organic C from agricultural soils. Alongside contributing to climate change via  $CO<sub>2</sub>$  emissions (Lal, [2004\)](#page-13-0), this depletion also compromises the essential foundation of soil structure and nutrient cycling, heralding a decline in soil fertility and health. As soil structure and function become disrupted, so does the equilibrium sustaining productive and resilient agricultural systems (Rabot et al., [2018\)](#page-14-1).

There is a growing need to rejuvenate arable soils and pave the way for sustainable agriculture (Rhodes, [2017\)](#page-14-2). Increasing the C content in arable soil offers numerous benefits with significant implications for agricultural productivity and environmental sustainability. Higher C levels enhance soil fertility by improving soil water-holding capacity, nutrient retention and microbial activity (Droste et al., [2020\)](#page-12-0), increasing crop yields and improving resilience against drought and nutrient deficiencies. Furthermore, increased C content enhances soil structure and stability, reducing erosion and promoting long-term soil health (Lal, [2016\)](#page-13-1). Several strategies for increasing soil carbon content in arable land have been effective, such as adopting conservation tillage, cover cropping and crop rotation (Crystal-Ornelas et al., [2021](#page-12-1); Laamrani et al., [2020](#page-13-2)). These rely on minimizing soil disturbance and partial organic matter retention within the soil. Alternatively, soil C can be increased by applying organic amendments such as compost, manure or biochar (Bong Cassendra Phun Chien et al., [2021](#page-12-2); Fischer & Glaser, [2012](#page-12-3); Liu, Zhang, et al., [2016](#page-13-3)).

A key determinant of soil recovery is increasing its content of stable or recalcitrant C compounds, which can be achieved by the judicious application of soil organic amendments. Amongst myriad such amendments, biochar, from pyrolysis of woody and other waste materials, is an obvious candidate (Lehmann & Joseph, [2009](#page-13-4)) because of the highly stable forms of carbon present. Biochar also has a highly porous structure that can retain water, nutrients and beneficial microorganisms in the soil (Abukari et al., [2022\)](#page-11-0) and residual ashes, which can induce a short-term liming effect on acidic soils, helping to increase the soil's pH buffering capacity (Bolan et al., [2022;](#page-12-4) Yoo et al., [2020\)](#page-14-3). Therefore, biochar has improved plant growth and nutrient uptake (Cao et al., [2019;](#page-12-5) Latini et al., [2019](#page-13-5); Sun et al., [2022](#page-14-4)).

By increasing nutrient availability, biochar can stimulate soil microbial activity and respiration, increasing microbial biomass (Rahman et al., [2020](#page-14-5)), including arbuscular

#### **Highlights**

- Complex biochar microgranules were tested under field and laboratory experiments.
- Biochar granulation is an efficient means of introducing mycorrhiza and other beneficial microbes.
- Mycorrhization of crop roots significantly improved with biochar supplementation.
- Microorganism coatings open exciting avenues for future investigations in sustainable agriculture.

mycorrhizal fungi (Lehmann et al., [2011](#page-13-6)). Soil conditions amended by biochar result in different effects on microbial community structure and diversity (Wu et al., [2017](#page-14-6)), for example, changes in the ratio of fungal and bacterial biomass (Igalavithana et al., [2017;](#page-13-7) McCormack et al., [2019\)](#page-13-8).

The availability of nutrients to plants can, however, be restricted by nutrient adsorption by freshly produced biochar or those made at high temperatures (El-Naggar et al., [2019](#page-12-6)), thus decreasing the crop yield (Schmidt et al., [2015\)](#page-14-7) as soil nutrient availability is directly reflected in plant biomass and yield (Oshunsanya et al., [2019\)](#page-13-9). The effectiveness of biochar may also vary depending on factors such as soil type, biochar quality, application rates and management practices. Biochar application in soils with low pH, low moisture content and low content of nutrients can significantly increase root biomass (Xiang et al., [2017\)](#page-14-8).

Two principal difficulties arise when using biochar to enhance soil C content and fertility in arable systems. First, most biochars do not contain sufficient levels of nutrients (Chen et al., [2019](#page-12-7)), and their application may result in excessive immobilization of nutrients already present in the soil (Brtnicky et al., [2021](#page-12-8); Joseph et al., [2021\)](#page-13-10). Second, biochar application in field conditions is challenging because of the powdery nature and irregular particle size of most products. Effective application rate control is especially difficult under dry and windy conditions. To address this, biochar can be manufactured as granules (Novak et al., [2014;](#page-13-11) Wang et al., [2022\)](#page-14-9), which can be improved by chemical and biological additives to create a high-impact product. Biochar granules can be enriched with nutrients such as nitrogen (N), phosphorus (P) and potassium (K) (Biederman & Harpole, [2013](#page-12-9)), with bacteria, and they can be inoculated with arbuscular mycorrhizal fungi (AMF) (Diagne et al., [2020](#page-12-10)). AMF can colonize most crop species and use the pores in biochar particles as colonization space protected from wider soil, at least initially (Warnock et al., [2007\)](#page-14-10). Despite the many benefits of granulating and producing bespoke biochar products, a paucity of field testing limits their deployment.

The objective of our study was to evaluate the effect of different biochar-based materials on the fertility of farmland soils. We conducted a series of controlled greenhouse and field studies to test the effectiveness of granulated and powdered biochars combined with other additives to improve crop plant growth and yield. We hypothesized that (H1) biochar addition to the soil will increase crop biomass and yield, (H2) biochar will alleviate the negative effect of drought on crop production and (H3) the combination of biochar and AMF will be the most effective solution to improve crop plant nutrition and productivity. Our study addresses the practical aspects of biochar application to soil, such as the lack of nutrients in pristine biochar, issues with its application because of the dusty nature of biochar and targeted application in precision agriculture.

### **2** | **MATERIALS AND METHODS**

The following methodology was designed to capture the multi-faceted impact of biochar addition to soil, involving soil chemical, biological and physical aspects: (i) the interaction between biochar and arbuscular-mycorrhizal fungi (AMF) under drought in a pot experiment, (ii) the impact of the size of biochar particles, microgranule versus powder, in a pot experiment and (iii) the effects of microgranule biochar on crop production in both pot and field settings.

#### **2.1** | **Soil collection and biochar(s) preparation**

Our experiments utilized two different soils from the Czech Republic: agricultural soil from a drought-prone site in Zveřínek (GPS: 50.1555567N, 15.0114297E) and

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a moderately dry brown earth sampled in Průhonice Botanical Garden (GPS: 49.9909092N, 14.5667783E). In both cases, the sandy and well-draining nature of these soils suggests an immediate advantage of biochar addition upon soil moisture retention. All soil samples were taken from the top horizon (0–20cm), using a W-shaped sampling design to obtain representative composite samples. Soil samples were air-dried, sieved (<2mm) and stored for 20days before starting the experiments. Soils characterized by basic chemical properties are shown in Table [1.](#page-4-0)

Two biochar products, both created from softwood feedstock, were used as soil amendments. Both products are registered as soil amendments by the Central Institute for Supervising and Testing in Agriculture, CZE. The powdered biochar (registered soil additive (Central Institute for Supervising and Testing in Agriculture, CZE), registration number 4867, NATURE CARBON) was produced through the gasification of raw wooden chips heated for 6h between 500 and 600°C (Brynda et al., [2020\)](#page-12-11). The gasification was done at a combined heat and power (CHP) plant in a fixed-bed multi-stage gasifier (GP750). MicroCHAR® (Aivotec s.r.o. and Groown s.r.o.) was produced according to patent No. 309512, where biochar obtained by thermal reduction of softwood at 550°C was crushed into particles of size 1–15mm (Maroušek et al., [2023](#page-13-12)). A mixture of biochar, poultry manure dry matter, bacteria of the genera *Rhizobium*, *Azotobacter*, *Pseudomonas* and *Bacillus* and spores of arbuscular mycorrhizal fungi and *Trichoderma* was prepared in the mixing device. The mixture was supplemented with a suspension of starch and boiling water and transported through a homogenizer into the granulating press, where granules with a diameter of 3mm were formed. The finished granules were then freed from scrapings in a vibration sorter and transported through a low-temperature



Abbreviations: PH soil, soil sampled in Průhonice; ZV soil, soil sampled in Zveřínek.

<span id="page-4-1"></span>a Values in brackets are available concentrations.

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dryer to a storage tank. This study combined the amendments with a range of other amendments via the following experimental scenarios (2.2).

#### **2.2** | **Experimental scenarios and setup**

#### 2.2.1 | Biochar and AMF as drought alleviaion measure

In this pot study, we used a full-factorial design with two levels of biochar addition and two levels of inoculation to describe the effect of biochar and mycorrhiza. We used the drought-prone soil collected from Zveřínek, setting up four treatments in total: (i) Zveřínek soil (ZV), (ii) Zveřínek soil inoculated with AMF  $(ZV+I)$ , (iii) Zveřínek soil with  $2\%$  (w/w) biochar (ZV+BC) and (iv) Zveřínek soil with 2% (w/w) biochar and inoculated with AMF  $(ZV + BC + I)$ . In respective treatments, biochar in powder form was mixed with dried soil and, where relevant, inoculated with 0.1 g of concentrated *Glomus intraradices* spores (on diatomite powder carrier 5000 spores per gram). Immediately after substrate preparation, six seeds of *Zea mays* were sown into each pot. The number of seedlings was reduced to four per pot after germination, aiming to remove the weakest and the strongest individuals. The reduction achieved greater homogeneity of plant material, sufficient rooting space for all plants and left a safety margin in case of plants dying.

The experiment was run in a greenhouse for 24weeks, after which the plants were harvested and the soil sampled. Pots were watered twice weekly with 100mL of water reduced to 25mL from week 19 to simulate a drought stress event. At the end of the experiment, all aboveground biomass was collected, dried (72h at 50°C) and weighed. Root samples from each pot were collected, stored at 4°C and analysed for mycorrhization using Trouvelot's method (Trouvelot et al., [1986](#page-14-11)). The elemental composition of harvested maize was determined using a TNM-L segment flow analyser (C and N) and by acid digestion, followed by ICP-OES (720 ES, Varian Inc., CA. USA) analysis (P and K). Elemental data were used to calculate C/N, C/P and N/P ratios. Soil samples were collected at harvest to determine nutrient availability (Ca, K, Mg, Mn, Na, P, S) using the Mehlich III extraction method followed by ICP-OES measurement.

## 2.2.2 | MicroCHAR<sup>®</sup> testing in the laboratory

**Soil incubation experiment (a)**: The Zveřínek soil (ZV) was amended with  $2\%$  (w/w) MicroCHAR® (ZV+MC) as treatment, and unamended soil was used as control. Soil mixtures were placed in 50mL closed tubes (*N*=5) and watered under field capacity level of ≈ −10kPa, calculated from the retention curve of this soil (Seyedsadr et al., [2022\)](#page-14-12). Soil pore water was collected by rhizons (Eijkelkamp, NL) and sampled at regular intervals for 20weeks: week 1, week 2, week 3, week 4, week 5, week 6, week 7, week 9, week 11, week 15 and week 20. Samples were analysed for dissolved organic carbon using a TOC-L a (CPH/CPN, Shimadzu), and macronutrient contents, that is, nitrogen (N), phosphorus (P) and potassium (K) using a TNM-L segment flow analyser (Shimadzu) and ICP-OES (720 ES, Variant Inc., CA, USA). **Greenhouse experiments (b):** The Zveřínek soil

(ZV) was amended with  $2\%$  (w/w) of biochar (ZV+BC) or MicroCHAR<sup>®</sup> ( $ZV+MC$ ) to compare the two char treatments, while soil without any amendment was used as control  $(n=5)$ . Soil with either char was placed in  $0.5L$ pots, and three *Pisum sativum* seeds were sown per pot. Plants were grown for 71days in a greenhouse with regular watering (100mL per week to ensure the same field capacity conditions as before), after which they were harvested, dried and weighed. The elemental composition of harvested peas was determined using the devices described above for the first incubation experiment and used to calculate element ratios in plants.

Průhonice soil (PH) was amended with MicroCHAR® only but at two doses. Three treatments were tested in this case: (i) soil without amendment (PH), (ii) soil with MicroCHAR<sup>®</sup> (PH+MC1) at 0.2g.pot<sup>-1</sup> and (iii) soil with MicroCHAR<sup>®</sup> (PH+MC2) at 2g.pot<sup>-1</sup>. MicroCHAR<sup>®</sup> was placed in shallow soil around the planted seeds. In total, 15 pots per treatment were filled with 1L of PH soil over a sterile textile layer to prevent soil loss. Three crop species were sown in this experiment, resulting in five pots per species per treatment: maize (two seeds per pot), wheat (five seeds per pot) and pea (two seeds per pot). Six days after germination, seedlings were reduced to one seedling per pot for corn and pea and four for wheat. Pots were regularly watered to keep the soil close to field capacity. Peas were harvested after 58days, wheat after 61days and corn after 111days, when all plants reached yield maturity. Plants were analysed for the dry weight of the grain, and root samples were collected to measure mycorrhization. In addition, wheat grain was subjected to nutrient content analysis by acid digestion, followed by ICP-OES analysis.

#### 2.2.3 | MicroCHAR<sup>®</sup> under field conditions

Finally, a field experiment was performed on the Průhonice Botanical Garden soil in 1×1.5m experimental plots. In total, 24 plots were prepared, and the following two treatments were randomly allocated: (i) soil with no amendment (PH) and (ii) MicroCHAR® (PH+MC). The amendment was applied at  $80 \text{ kg.ha}^{-1}$  (50g per experimental plot), representing a dose within the 0.2 and 2 g per plant rate as in the pot experiment above. A random half of the plots was allocated to wheat, while the other half was for maize. The MicroCHAR® was applied in the sowing row for wheat (five rows with 4.5 g of evenly spread seeds in each experimental plot) and around individual seeds for corn (four rows, six seeds per row in each experimental plot). The crops were watered regularly for a week to ensure the germination of planted seeds, after which all plants were grown for 105days and harvested and analysed for grain dry weight and mycorrhization. In addition, the soil was collected in each block (in a W shape); the samples of the same treatment were pooled to make a representative sample and analysed for key chemical properties, that is, pH, total N and its forms, organic C and total and available nutrients (P, Ca, Mg, K).

#### **2.3** | **Statistical analysis**

All data were analysed using R. Data were analysed separately for each species. In each case, the normality of the data was evaluated using the Shapiro test, followed by a Bartlett or Fligner test to evaluate their homoscedasticity. Finally, means were compared using the ANOVA test (homoscedastic data) or the Kruskal–Wallis test (non-homoscedastic data), followed by a post hoc test. After verification of the normality of the data, the incubation dataset was evaluated by repeated measures ANOVA. Finally, the data from pot

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and field experiments were pooled together to evaluate the overall influence of biochar materials on crop yield and mycorrhization using linear mixed-effects models. Treatment effects were considered significant at *p*<.05.

### **3** | **RESULTS**

### **3.1** | **Biochar and AMF as drought alleviation measures**

Soil nutrient availability was determined at the end of each experiment to evaluate the intensity of nutrient depletion (Table [2](#page-6-0)). Most of the nutrients were affected by amendments; potassium was increased by all three treatments ( $p < .001$ ); calcium availability was reduced by inoculation  $(p < .001)$  but not when biochar and inoculum were combined, while biochar alone increased it (*p*<.05); magnesium availability was increased by the combined treatment  $BC+I$  ( $p < .001$ ); manganese availability was reduced by the inoculation treatments, alone  $(p < .001)$ but increased in combination with biochar (*p*<.001); sodium availability decreased with the inoculum treatments (*p*<.01) and sulphur availability only increased when biochar was added alone  $(p < .01)$ . Crop plant biomass was not significantly affected by biochar or inoculum addition (*p*=.396, Table [3](#page-6-1)). Adding either biochar or AMF increased the grain's nutrient content  $(p < .001$  for N, P, K and C) without a synergistic effect when applied together.

<span id="page-6-0"></span>**TABLE 2** Soil nutrient availability (mg.kg−1) after 24weeks of maize growth on the different substrates.

	Calcium	<b>Potassium</b>	<b>Magnesium</b>	<b>Manganese</b>	Sodium	<b>Phosphorus</b>	Sulphur
ZV	$511 + 27 b$	$45 + 3d$	$60 + 3 b$	$65 + 3 b$	$98 + 21a$	$325 + 18a$	$29 \pm 3$ b
$ZV+I$	$387 + 10c$	$58 + 2c$	$59 + 1 b$	$57 + 1c$	$64 + 11 b$	$307 + 5a$	$25\pm 8$ b
$ZV + BC$	$556 + 29a$	$64 + 3 b$	$61 + 3 b$	$61 + 3$ bc	$80 + 6a$	$328 + 15a$	$44 \pm 5$ a
$ZV + BC + I$	$513 + 18 b$	$93 + 4a$	$68 + 1a$	$89 + 2a$	$68 + 5 b$	$320 + 7a$	$30 + 4 b$

*Note*: Letters indicate significant difference  $(n=5)$   $(p < .05)$ .

Abbreviations: BC+I, Zveřínek soil amended with 2% of biochars (w/w) and inoculated with *Glomus intraradices*; BC, Zveřínek soil amended with 2% of biochar (w/w); ZV+I, Zveřínek soil inoculated with *Glomus intraradices*; ZV, non-amended Zveřínek soil.

<span id="page-6-1"></span>**TABLE 3** Grain biomass, element concentrations and C/N, C/P and N/P ratios measured in the maize plants grown on the different substrates.

	Grain biomass $(g)$	Carbon $(g \, kg^{-1})$	<b>Nitrogen</b> $(g \, kg^{-1})$	<b>Phosphorus</b> $(g \, kg^{-1})$	Potassium $(g \, kg^{-1})$	C/N	C/P	N/P
ZV	$6.6 + 1.3a$	$451 + 4 b$	$10.1 + 0.2$ c	$1.50 + 0.56$ b	$4.37 + 1.18$ b	$45 + 1a$	$330 + 104$ a	$7.3 + 2.2a$
$ZV+I$	$6.6 + 3.2a$	$462 + 1a$	$13.0 + 0.3$ a	$3.27 + 0.42$ a	$9.48 + 0.94$ a	$35 + 1c$	$143 + 18 b$	$4.0 + 0.5 b$
$ZV + BC$	$8.5 + 0.9 a$	$446 + 5 b$	$11.8 + 0.2 b$	$3.36 + 0.18$ a	$10.48 + 0.86$ a	$38 + 1 b$	$133 + 6 b$	$3.5 \pm 0.1$ b
$ZV + BC + I$	$6.7 + 2.0$ a	$451 + 3 b$	$9.9 + 0.3$ c	$2.92 + 0.69$ a	$9.42 + 2.22$ a	$46 + 1a$	$161 + 6 b$	$3.5 + 0.7 b$

*Note*: Letters indicate significant difference  $(n=5)$  ( $p < .05$ ).

Abbreviations: BC+I, Zveřínek soil amended with 2% of biochars (w/w) and inoculated with *Glomus intraradices*; BC, Zveřínek soil amended with 2% of biochar (w/w); ZV+I, Zveřínek soil inoculated with *Glomus intraradices*; ZV, non-amended Zveřínek soil.

Improvement of soil fertility because of biochar addition typically leads to reduced C/N and C/P ratios in the plant; this study confirmed this effect for both ratios  $(p < .001)$ . The value of the N/P ratio decreased because of the application of either biochar or AMF (*p*<.01 for inoculum alone and  $p < .001$  for biochar and biochar+inoculum). The inoculation treatment alone did not enhance the mycorrhization of the corn plant roots ( $p = .15$  $p = .15$  $p = .15$ , Figure 1). Still, biochar increased mycorrhization (*p*<.05) with no additional effect of AMF inoculation.

ab

## **3.2** | **MicroCHAR as an all-in-one soil amendment**

## 3.2.1 | Incubation experiment

a

The amount of organic carbon dissolved in the soil solution peaked after applying MicroCHAR but later decreased and was not significantly different from the control (Figure [2a](#page-7-1)). Availability of all three assessed macronutrients such as N, P and K was increased by

> <span id="page-7-0"></span>**FIGURE 1** Mycorrhization of maize plants grown on the different substrates. BC+I, Zveřínek soil amended with 2% of biochars (w/w) and inoculated with *Glomus intraradices*; BC, Zveřínek soil amended with 2% of biochar (w/w); ZV+I, Zveřínek soil inoculated with *Glomus intraradices*; ZV, non-amended Zveřínek soil. Letters indicate significant difference  $(n=5)$  ( $p < .05$ ).



a

<span id="page-7-1"></span>**FIGURE 2** Soil pore water dissolved organic carbon (a), nitrogen (b), phosphorus (c) and potassium (d) concentrations (mgL−1) measured during 20weeks in the Zveřínek soil (ZV) amended with 2% (w/w) Microchar (ZV+MC). Letters indicate significant difference within each week  $(n=5)$   $(p < .05)$ .

100

80

 $20$ 

Mycorrhization (%)



<span id="page-8-0"></span>**FIGURE 3** Biomass production (root, shoot, pea) (a) and pea element concentrations (C (b), N (c), P (d) and K (e)) of pea plants grown on the different treatments. ZV+BC, Zveřínek soil amended with 2% (w/w) biochar; ZV+MC, Zveřínek soil amended with 2% (w/w) Microchar; ZV, non-amended Zveřínek soil. Letters indicate significant difference within each week (*n*=5) (*p*<.05).

<span id="page-8-1"></span>



*Note*: Letters indicate significant difference within each week  $(n=5)$  $(p < .05)$ 

Abbreviations: ZV+BC, Zveřínek soil amended with 2% (w/w) biochar; ZV+MC, Zveřínek soil amended with 2% (w/w) Microchar; ZV, nonamended Zveřínek soil.

applying MicroCHAR (*p*<.001). The increase in P availability was consistent over the entire incubation time (Figure [2c\)](#page-7-1) but was only temporary for N (Figure [2b\)](#page-7-1) and K (Figure [2d\)](#page-7-1).

#### 3.2.2 | Greenhouse experiments

Greenhouse experiments were conducted to compare MicroCHAR with standard biochar on the Zveřínek soil. MicroCHAR had a positive effect on root biomass and pea P content in pea plants (Figure [3](#page-8-0)) when compared with control  $(p < .001)$  and biochar  $(p < .001)$ . As a result, C/P and N/P ratios in peas grown in soil enhanced with MicroCHAR were reduced (*p*<.001 for C/P and *p*<.05 for N/P), hinting at better P nutrition (Table [4](#page-8-1)). However, neither biochar nor MicroCHAR enhanced N (*p*>.05) or  $K(p > .05)$  content in peas.

In a second pot experiment, two application doses of MicroCHAR were tested on the second soil (Table [S1](#page-14-13)).

Maize cob biomass was not increased as a result of the MicroCHAR application. Quite to the contrary, the lower dose decreased cob weight (*p*<.05). However, the dry weight of cobs produced by wheat and peas was unaffected  $(p > .05)$ . Similarly, the amendments did not significantly increase the nutritious state of wheat grain at either dose rate. However, a positive effect was observed for the mycorrhization rate of the roots, which significantly increased in the case of the high dose for peas  $(p < .04,$  $(p < .04,$  $(p < .04,$  Figure 4).

#### 3.2.3 | Field testing

Field application of MicroCHAR did not affect wheat and maize yield  $(p > .05,$  Figure [S1\)](#page-14-13). However, the rate of mycorrhization was increased in both crops because of adding this product to the soil  $(p < .01,$  Figure [5\)](#page-9-1). Soil samples were taken from the control, and plots were amended at the end of the field experiment. Soil pH was decreased by the MicroCHAR treatment (Table [S2](#page-14-13)) because of biochar activation via its surface oxidation and presence of acidifying nutrients. Total N content, total C content and total organic C content were increased as a result of the MicroCHAR application. The concentrations of available ammonium-N decreased in the amended plots while the available content of nitrate-N increased. All the other measured elements (P, K, Ca, Mg), in total and available concentrations, were higher in the amended conditions than the control, except for K. In the case of K, total concentration was not affected, while available concentration decreased in plots amended with MicroCHAR.



<span id="page-9-0"></span>**FIGURE 4** Mycorrhization (%) measured on the roots of pea (a), wheat (b) and maize (c) under the different treatments, under greenhouse conditions. MC1, Průhonice soil amended with 0.02% (w/w) of MicroCHAR; MC2, Průhonice soil amended with 0.2% (w/w) of MicroCHAR; PH, non-amended Průhonice soil. Letters indicate significant difference  $(n=5-15)$   $(p < .05)$ .

<span id="page-9-1"></span>**FIGURE 5** Mycorrhization (%) measured on the roots of wheat (a) and maize (b) under the different treatments, under field conditions. PH+MC, Průhonice soil amended with MicroCHAR; PH, non-amended Průhonice soil. Letters indicate significant difference (*n*=3–9)  $(p < .05)$ .

#### **3.3** | **Overall effect of the biochar addition**

Data from the different pot and field experiments were pooled to evaluate the overall effect of biochar addition on crop yield and mycorrhization, which were used as plant performance indicators in this study. Crop yield was not significantly affected (*p*>.05), while mycorrhization was significantly enhanced by biochar addition to the soil (*p*<.001).

## **4** | **DISCUSSION**

#### **4.1** | **Benefits of biochar-based materials on soil fertility**

Incorporating biochar, whether in powder or microgranule form, affected soil chemistry and microbial characteristics. The elevation in soluble, total and available nutrient concentrations confirms this. Notably, the increase was detectable even after the rapid crop growth and nutrient uptake phase, indicating that the reservoir of nutrients enhanced by the biochar materials was not exhausted by the demands of plant growth. These findings are in agreement with earlier empirical observations (El-Naggar et al., [2015;](#page-12-12) Kamran et al., [2018;](#page-13-13) Karimi et al., [2020](#page-13-14)) and are driven by the nutrient contents of amended biochars, which are typically higher than those of the soils (Table [1;](#page-4-0) Chintala et al., [2014](#page-12-13); Curaqueo et al., [2021](#page-12-14)). Furthermore, our results underscore the sustained efficacy of biochar-based amendments, particularly in their microgranule form. Notably, N, P and K continued to be released even after 20 weeks and remained available after the completion of plant growth in the field. This durability suggests the potential for prolonged effectiveness across cropping seasons without necessitating frequent reapplication.

In addition to the marked impact on nutrient availability, our study revealed a discernible alteration in the soil microbial community because of these amendments, as evidenced by the strong increase in root mycorrhization. Greenhouse experiments demonstrated the positive effect of powdered biochar on root mycorrhization, while MicroCHAR in higher doses significantly increased mycorrhization of peas in pots and wheat and maize grown in the field. Biochar may, thus, be an effective vehicle for introducing beneficial microbes into the soil. Mycorrhiza, but also microorganisms involved in essential nutrient cycling processes, such as (de)nitrification, phosphate solubilization and carbon and nitrogen mineralization (Enaime & Lübken, [2021](#page-12-15); Yadav et al., [2021](#page-14-14)) may be positively affected by biochar addition. The increase in

abundance and activity of soil microorganisms likely contributed to the overall enhancement in nutrient availability. Long-term release of nutrients, as well as enhancement of AMF, are in accordance with previous studies, which demonstrated increased water retention, better nutrient retention and enhanced microbial activity (Lebrun et al., [2022](#page-13-15), [2024;](#page-13-16) Seyedsadr et al., [2022\)](#page-14-12). This study's findings hold particular importance in the context of N and P, two elements often deficient in arable soils. The observed change in the N/P ratio in plant tissues after applying biochar-based materials suggests mitigation of P limitation (Cao & Chen, [2017;](#page-12-16) Xu et al., [2022](#page-14-15)). Biochar addition may also affect microbial communities negatively, especially if unadulterated (not moistened/oxidized) biochar is used. A desiccation effect is likely to occur, with biochar absorbing all water from the soil (Hagemann et al., [2017](#page-13-17)).

In the context of addressing carbon depletion in arable soils, our study confirms the beneficial effects of biochar. The total carbon content increased in all biochar addition treatments; a phenomenon likely attributed to the substantial carbon content present in the amendments. Concurrently, the dissolved organic carbon content was reduced, in alignment with prior investigations (Bohara et al., [2019;](#page-12-17) Hailegnaw et al., [2019;](#page-13-18) Rombolà et al., [2022\)](#page-14-16). The decrease in dissolved organic carbon (DOC) levels in soil after adding biochar can be attributed to several interconnected processes: adsorption and desorption because of the high surface area and a porous structure of biochar (Kumar et al., [2020](#page-13-19)), immobilization by microorganisms colonizing biochar and consuming DOC as a carbon source (Thies & Rillig, [2009](#page-14-17)) or by chemical stabilization in stable complexes between DOC and biochar (Liu et al., [2018\)](#page-13-20). Finally, beyond this study's temporal frame of reference, biochar is considered relatively stable. It can persist in the soil for a significant period, potentially hundreds to thousands of years (Gurwick et al., [2013](#page-12-18)). Adding meaningful amounts of biochar to arable soils can, thus, have a long-term positive effect by increasing their C content for a considerable time.

The effects of biochar also appear universal. In light sandy soils with a limited amount of organic matter, biochar leads to improved soil water and nutrient retention (Lebrun et al., [2022](#page-13-15), [2024](#page-13-16); Seyedsadr et al., [2022](#page-14-12)), whereas in heavy clay soils, biochar aids aeration and increases soil permeability by lowering bulk density.

#### **4.2** | **Benefits of biochar for the plants**

In this study, biochar addition also demonstrated positive effects on plant performance. While it did not lead to a significant increase in biomass production, its addition enhanced the nutritional status of the crops. Numerous

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studies have highlighted the capacity of biochar to improve crop growth (Curaqueo et al., [2021;](#page-12-14) Gonzaga et al., [2021](#page-12-19); Sun et al., [2021\)](#page-14-18). This improvement can be attributed to the alteration of soil physicochemical properties, including a reduction in bulk density (Liang et al., [2019\)](#page-13-21), increased water retention (Lebrun et al., [2022\)](#page-13-15) and nutrient availability (Curaqueo et al., [2021](#page-12-14); Khan et al., [2020\)](#page-13-22). Seyedsadr et al. [\(2022\)](#page-14-12) pointed out that the high porosity, especially the volume of mesopores, and very high Brunauer–Emmett–Teller (BET) surface area result in a substantial ability of biochar to hold and slowly release pore water together with dissolved elements.

Our findings corroborate the positive influence of biochar on soil nutrient availability. However, it is noteworthy that this enhancement did not yield a significant increase in plant growth. The crop yield of wheat and peas in the greenhouse experiment and maize and wheat in the field experiment were not changed, while the application of MicroCHAR at a lower dose decreased maize cob weight in the greenhouse experiment. Pinto et al. [\(2023](#page-14-19)) report no positive effect of biochar on sugar cane plant development, while biochar positively influenced soil bacteria. This outcome may be linked to two plausible factors: firstly, the nutritional status of the soil may have already been optimal for the crop (Seehausen et al., [2017](#page-14-20); Védère et al., [2023](#page-14-21)); and secondly, nutrient availability in wider soil may not have reached the threshold sufficient to saturate nutrient retention within biochar structures (Farhangi-Abriz et al., [2021\)](#page-12-20).

Introducing biochar materials into the soil led to a discernible increase in the levels of essential macronutrients, such as N, P and K, within the plants. These findings align with prior observations (Cao et al., [2019](#page-12-5); Liu, Lu, et al., [2016](#page-13-23)) and can be attributed to the increased nutrient content and improved availability in the amended soil substrates. The augmentation of nutrient content in plants holds significant importance as these nutrients play pivotal roles in sustaining the metabolic processes of plants. For instance, K contributes to sugar production (Altay & Aksu, [2020;](#page-11-1) Hasanuzzaman et al., [2018\)](#page-13-24), while P is essential for energy transfer, enzyme activity regulation and photosynthesis (Amtmann et al., [2005](#page-12-21); Ashkevari et al., [2013](#page-12-22); Touchette & Burkholder, [2000](#page-14-22)). These observations underscore that, even though there was no substantial increase in biomass production, the overall quality of the crops was enhanced, enhancing human nutrition. This effect was more pronounced under drought stress conditions, especially concerning N and K, highlighting the efficiency of biochar when applied to degraded soils or in challenging environmental settings (Jeffery et al., [2017;](#page-13-25) Schmidt et al., [2021\)](#page-14-23).

When looking at plant symbionts, it became evident that biochar played a pivotal role in enhancing plant root mycorrhization, thereby showcasing its dual capacity as

### both a stimulant for the native AMF community and a vehicle for introducing exogenous AMF strains. Several factors can account for the observed enhancement in mycorrhization. Firstly, by elevating nutrient availability, biochar not only expands the nutrient reservoir accessible to plants but also enriches the nutrient pool available to other macro- and microorganisms residing in the soil (de Figueiredo et al., [2019\)](#page-12-23). Secondly, the porous structure of biochar serves as a conducive habitat and sanctuary for the fungal hyphae, promoting their growth and development (Curaqueo et al., [2021](#page-12-14)). Lastly, previous studies have demonstrated biochar's ability to reduce bulk density and enhance soil aeration, which is highly beneficial to microorganisms (dos Santos Trentin et al., [2022](#page-12-24)).

#### **4.3** | **Benefits of the biochar granulation**

Our research findings underscore the efficacy of biochar, whether in powder or microgranule form, in enhancing soil fertility and promoting the colonization of roots by AMF. MicroCHAR®, perhaps owing to higher nutrient concentrations than traditional biochar, could be applied in significantly lower dosages in most scenarios and still support plant growth. Furthermore, the microgranular size of MicroCHAR®, akin to crop seeds, allows for precise and localized application, enabling it to be placed near plant seeds. This precision reduces costs and leverages existing farm machinery, further economizing the process and simplifying application logistics. Additionally, MicroCHAR® can be an ideal carrier for fertilizers, facilitating the precise delivery of essential nutrients to the root systems. This dual functionality minimizes the expense of fertilizer procurement and application and the risk of nutrient leaching. During the production of MicroCHAR®, it becomes possible to incorporate beneficial microorganisms, such as AMF or phosphorus solubilizing bacteria, onto the granules. This integration offers added advantages, particularly concerning nutrient uptake. Consequently, a modest amount of MicroCHAR® can be periodically introduced into the soil each year, gradually accumulating organic matter and nutrient-storing capacity. Applying such complex biochar granulates (containing nutrients and all the promoting microorganisms) could soon substitute conventional fertilizers as a sustainable bio-based solution.

Given that the addition of enhanced biochars has broadly positive effects on the soil–crop plant system, a cost–benefit analysis should be undertaken. Firstly, compared with pristine biochar and conventional mineral fertilizers, granulated biochar with additives represents more costly slow-release organic fertilizer. The higher price of microgranule fabrication may be compensated by targeted

application in precision agriculture where the amount of required biochar can be significantly reduced. Secondly, additional long-term benefits may arise from combining biochar with AMF inoculants. A complete cost–benefit analysis is necessary and constitutes an interesting area of future research.

#### **5** | **CONCLUSION**

This study has highlighted the substantial potential of biochar in enhancing soil fertility, even when applied in minimal doses, while concurrently elevating the nutritional quality of crops. It has shown the synergistic possibilities of combining biochar with microorganisms, particularly AMF. Notably, the utilization of biochar microgranules enriched with AMF emerged as an efficient vector for introducing mycorrhizal inocula and potentially other beneficial microbes. However, the benefits to plants were not universally consistent, warranting further research to determine the optimal composition and dosage for various crop species. Additionally, exploring the combined coating of nutrients and microorganisms on MicroCHAR® represents an exciting avenue for future investigations.

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#### **DATA AVAILABILITY STATEMENT**

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

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## <span id="page-14-13"></span>**SUPPORTING INFORMATION**

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