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# Carbon-enriched organic amendments differently affect the soil chemical, biological properties and plant biomass in a cultivation time-dependent manner

J. Holatko<sup>1,2</sup>, T. Hammerschmiedt<sup>1</sup>, A. Mustafa<sup>1,3,4\*</sup>, A. Kintl<sup>1,5</sup>, M. Radziemska<sup>1,6</sup>, T. Baltazar<sup>1</sup>, I. Jaskulska<sup>7</sup>, O. Malicek<sup>1</sup>, O. Latal<sup>1,2</sup> and M. Brtnicky<sup>1,3\*</sup>

## Abstract

**Background:** The farmyard manure application maintains quality of arable soils, provides nutrients, mitigates climate change by soil carbon sequestration. Biochar and other complex carbon rich amendments may stabilize organic matter derived by composting and decelerate organic carbon mineralization. However, how the combined utilization of biochar, humic substances and manure effects on soil chemical and biological properties have been least explored, especially their effect on soil basal and substrate induced respirations are needed to be further explored. Therefore, the potential of biochar and Humac (a commercial humic substances product) in combination with manure to improve the soil properties and plant growth was investigated in this experiment using barley under a short-term (12 weeks) and maize under long-term (following 12 weeks, a total of 24 weeks) cultivation.

**Results:** In the early phase of cultivation (12 weeks) Humac- or biochar-enriched manures (M + H, M + B, respectively) enhanced the contents of nutrient elements (carbon + 5.6% and + 7%, nitrogen + 6.7% and – 5%, sulphur – 7.9% and + 18.4%), the activity of enzymes including ( $\beta$ -glucosidase + 32% and + 9.6%, phosphatase + 11% and 6.3%), and dry aboveground biomass (+ 21% and + 32%), compared to the control and manure-treated soil. However, these impacts of M + H and M + B manures were reduced under longer period, i.e., at the experiment end (24 weeks). After 24 weeks of cultivation, a decrease in absolute values of all determined enzyme activities indicated putative reduction of mineralization rate due to presumed higher recalcitrance of manure-derived organic matter, with Humac, biochar amendments. Increased stability of soil organic matter reduced microbial activity due to lower availability of nutrients. Possibly, the shortened period of manure maturation could help preserve a higher amount of less degraded organic matter in the enriched manures to counteract these observed features.

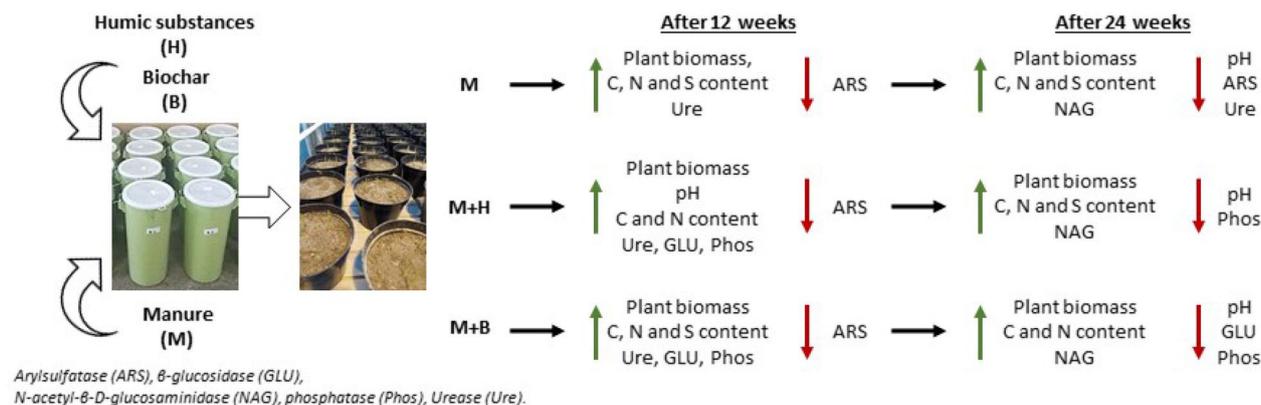
**Conclusions:** We summarized that the biochar and humic substances combined with manure have the potential to improve the soil characteristics, plant biomass and soil health indicators but the improvements faded away in a cultivation time-dependent manner. Further studies are required to explore the structure and functioning of microbial activities under long-term experimental conditions.

\*Correspondence: adnanmustafa780@gmail.com; martin.brtnicky@seznam.cz

<sup>1</sup> Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, 61300 Brno, Czech Republic  
Full list of author information is available at the end of the article

**Keywords:** Biochar, Humic acids, Manure, Maturation enrichment, Soil nutrients, Soil enzyme activity

**Graphical abstract**



**Background**

The world agriculture is facing critical issues those related to environmental degradation, climate change, and food security [1]. The losses of arable soil and the decrease in its fertility (i.e., reduced nutrient content, biological diversity and resilience to sustain environmental changes) are the main contemporary problems [2]. After several decades of intensification of agriculture production, in terms of the overestimated use of chemical fertilizers, the return to the more conventional types of organic fertilizers is seemingly a promising approach which could alleviate the degradation of soil and restore the soil fertility and sustainable crop yield [2]. The farmyard manure, one of the most common types of organic fertilizers maintain quality and healthy arable soils for the sustainable agriculture [3]. It provides a large source of nutrient elements, especially carbon, nitrogen, phosphorus, and minerals (e.g., potassium), beneficial for both, plants and the soil microbiota [3]. Manure-derived organic matter plays a positive role in climate change mitigation by soil carbon sequestration [4]. Positive effects of soil treatment with manure on total soil carbon, nitrogen, microbial biomass carbon, and dehydrogenase activity in soil has been generally observed [5, 6]. However, the time scale impacts on treated soil and its properties after application is limited by the rate of decomposition of complex compounds in the manure and availability and leachability of nutrients [4]. The manure enriched with more recalcitrant organic materials such as biochar had mostly positive effects on plant growth, crop productivity, soil microbial diversity and abundance when applied to soils [7–9]. The observed benefit of biochar was ascribed to its large specific surface, microporosity, and sorption capacity [10], which

can also affect composting process of agriculture waste materials, such as cattle manure [11]. Reported advantages of manure enriched during maturation with biochar included the mitigation of green-house gasses emission [12, 13] mainly ammonia [14], nutrition loss protection [15], modification of fermentation thermodynamics and heat formation [16], alteration and improvement of manure functional microbial diversity [17, 18]. Mitigation of ammonia emission is facilitated due to  $\text{NH}_4^+$  ion assimilation into organic compounds during microbial utilization of dissolved organic matter [19], which is a labile fraction of total soil organic matter. Its ratio to the recalcitrant carbon decreases with the higher pyrolysis temperature of the feedstock [20]. The technology of manure composted with biochar may prolong the fertilizing effects of both amendments due to the potential of biochar in slow release of nutrients and hence aiding in plant nutrition [21]. Biochar–manure mixture has been reported to improve total soil carbon, nitrogen, bulk density and impacted the availability of nutrient macro- and microelements differently compared to the sole manure treatment [22, 23]. It has been established that the short-term effects of biochar-compost increases the contents of macro- and microelements after soil application [24]. Yet, other studies revealed that composted matter stimulated the co-mineralization of less recalcitrant components of biochar over the short (<2 months) duration, while the biochar may later stabilize organic matter derived by composted matter and reduce OC mineralization [25]. Therefore, it was a call for further investigation if this effect may be detectable on the level of other soil properties and plant growth response. Concurrently, it is obvious that the extent and generality of this feature

for other types of recalcitrant carbon-rich amendments (such as humic substances), added to soil as a co-fertilizers and soil conditioners together with manure, has been underexplored. It is further known that long-term treatment of arable soil with manure enriches soil organic carbon (SOC) pool with humic substances which further depend on the manure quality and quantity and ratio of humic acids, fulvic acids and humins [26]. It was found that labile organic compounds are effectively altered in soil by humification, and their mineralization substantially reduced [27]. Thus, we intended to test the effect of externally applied humic acids on the process of manure maturation and verify if additional humic substances (via hydrophobic protection) can increase total soil carbon content and decelerate its mineralization.

Therefore, we designed a two-phase plant growth pot experiment aiming to evaluate the early (short-term) and the later (long-term, e.g., twice as long as the short-term) effect of manure and co-composted manure (with biochar or Humac = humic substances) single application on the soil chemical and biological properties under barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) cultivation in pots, which differs from conventional field practice when manure is applied periodically. Our main objective was to check how the soil properties change after shorter and longer interval (12 and 24 weeks, respectively) of treatment with different manure amendments. We consider as relevant that, this experimental approach unlike to conventional approach emphasizes the sole utilization of manure amendments and assessment of their effects would counteract the effects caused by repeated additions. We thus, hypothesized that the extent of change (magnitude of alteration) induced by enriched manure amendment will decrease in time and the presumed higher benefit of manure enriched with carbon additives (biochar or Humac) to the plant nutrition and microbial activity will be mitigated in time-dependent manner.

## Materials and methods

### Procurement and preparation of C-enriched manures

Fresh manure was obtained from suckler cattle bred on deep bedding. Carbon enriched-manures were prepared by two methods (a) by mixing of fresh manure with HA-rich product Humac AGRO (commercial soil

fertility stimulator, mined from leonardite–oxihumolite, provided by Envi Produkt Ltd., Prague, Czech Republic), and (b) by mixing of fresh manure with biochar produced from agricultural waste (cereal husks, sunflower peels and fruit processing waste) at 600 °C (Sonnenerde GmbH, Riedlingsdorf, Austria). Detailed information on the amendment has been previously published [28, 29].

Fresh manure and both additives were dosed into the 50-L sealable barrels (Table 1). Each variant was prepared in 3 barrels (replicates), that were tightly sealed to prevent desiccation. Activation process was carried out for 8 weeks at room temperature. The properties of prepared manure variants used for the pot experiment are in Table 2.

### Collection of soil, treatment plan and pot experiment

The soil was collected from the experimental field near the town Troubsko, Czech Republic (49°10'28"N 16°29'32"E). The soil was silty clay loam (according to USDA Textural Triangle), Haplic Luvisol (according to WRB soil classification), properties are described in detail by Brtnický et al. [29]. The topsoil was dug up to the depth of 15 cm, big parts (stones, plants residues) were removed on a 2 mm sieve, the sieved soil was then thoroughly mixed with a fine quartz sand (0.1–1.0 mm) in a weight ratio of 1:1.

The three tested variants were made by thorough mixing of 5 kg of experimental soil–sand mixture with 150 g of particular manure type per a pot, equals to 50 t ha<sup>-1</sup>. Unamended control contained only 5 kg of experimental soil–sand mixture. Each variant was prepared in four replicates and marked equally to the used manure type: (1) (unamended) control, (2) manure (M), (3) manure + Humac (M + H), (4) manure + biochar (M + B).

To check the efficacy of prepared variants, barley (*Hordeum vulgare* L.) was sown as a test crop in all pots. Each pot was sown with 16 barley seeds at 2 cm soil depth and was watered with distilled water to achieve 65% water holding capacity. This moisture level was maintained throughout the experiment. All pots were placed randomly into growth chamber (CLF Plant Climatics GmbH, Wertingen, Germany) and rotated once per week to ensure homogeneity of conditions for the treatments. Controlled conditions were set as follows:

**Table 1** Manure variants and additive dosage

Variant	Abb	Manure per barrel	Additive per barrel	Dry matter ratio M:additive	Moisture content of final manure
manure	M	10 kg	0	0	70.0%
Manure + humac	M + H	10 kg	0.1 kg	41:1	68.8%
Manure + biochar	M + B	10 kg	4.0 kg	1.25:1	64.0%

**Table 2** Properties of manure variants used for the pot experiment

	Dry matter [%]	pH	N_NO <sub>3</sub> [mg kg <sup>-1</sup> ]	N_NH <sub>4</sub> [mg kg <sup>-1</sup> ]	N_min [mg kg <sup>-1</sup> ]	Ash [%]
M	30.0078 ± 0.0002	9.035 ± 0.298	14.642 ± 0.482	2.055 ± 0.068	16.697 ± 0.550	18.200 ± 0.600
M + H	31.2235 ± 0.0002	9.245 ± 0.305	15.581 ± 0.513	1.561 ± 0.051	17.142 ± 0.565	24.598 ± 0.822
M + HB	36.0109 ± 0.0002	8.705 ± 0.287	11.750 ± 0.388	2.581 ± 0.085	14.331 ± 0.473	42.019 ± 1.385
	TC [%]	P [g kg <sup>-1</sup> ]	K [g kg <sup>-1</sup> ]	Mg [g kg <sup>-1</sup> ]	Ca [g kg <sup>-1</sup> ]	TN [%]
M	9.100 ± 0.300	4.220 ± 0.140	84.365 ± 2.786	9.607 ± 0.317	20.930 ± 0.690	2.479 ± 0.082
M + H	12.300 ± 0.411	3.577 ± 0.120	87.568 ± 2.888	9.302 ± 0.307	22.679 ± 0.748	2.490 ± 0.082
M + HB	21.010 ± 0.692	3.041 ± 0.101	85.482 ± 2.824	7.986 ± 0.263	159.335 ± 5.261	1.991 ± 0.066
	C_HS [%]	C_HA [%]	C_FA [%]	HA:FA	C_org [%]	Zn [mg kg <sup>-1</sup> ]
M	7.412 ± 0.244	3.265 ± 0.108	4.147 ± 0.137	0.787 ± 0.026	13.503 ± 0.445	91.569 ± 3.025
M + H	3.016 ± 0.099	1.641 ± 0.054	1.375 ± 0.045	1.196 ± 0.040	12.454 ± 0.411	94.573 ± 3.141
M + HB	1.993 ± 0.066	1.334 ± 0.044	0.659 ± 0.022	2.037 ± 0.067	11.894 ± 0.392	27.745 ± 0.924
	Fe [g kg <sup>-1</sup> ]	B [mg kg <sup>-1</sup> ]	S [%]	AOB [copie g <sup>-1</sup> ]	nirS [copie g <sup>-1</sup> ]	dsr [copie g <sup>-1</sup> ]
M	1.144 ± 0.038	47.20 ± 1.56	0.786 ± 0.026	21.1 ± 0.033 · 10 <sup>7</sup>	10.7 ± 0.020 · 10 <sup>8</sup>	10 ± 0.01 · 10 <sup>6</sup>
M + H	1.134 ± 0.037	43.78 ± 1.45	0.371 ± 0.012	10.1 ± 0.017 · 10 <sup>7</sup>	18.7 ± 0.029 · 10 <sup>8</sup>	150 ± 0.30 · 10 <sup>6</sup>
M + HB	1.054 ± 0.035	58.31 ± 1.92	0.327 ± 0.011	7.46 ± 0.007 · 10 <sup>7</sup>	18.5 ± 0.027 · 10 <sup>8</sup>	9.6 ± 0.01 · 10 <sup>6</sup>

Average values ( $n = 3$ ) ± standard error of mean

The properties displayed are: N\_NO<sub>3</sub>, nitrogen in nitrate form; N\_NH<sub>4</sub>, ammonium nitrogen; N\_min, mineral nitrogen; TC, total carbon; P, total phosphorus; K, total potassium; Mg, total magnesium; Ca, total calcium; TN, total nitrogen; C\_HS, carbon in humic substances; C\_HA, humic acid carbon; C\_FA, fulvic acid carbon; HA:FA, humic: fulvic acid ratio; Zn, total zinc; Fe, total iron; B, total boron; S, total sulphur; AOB, amount of ammonia oxidizing bacteria (detected as amoA marker gene copies); nirS, amount of denitrifying microorganisms (detected as nirS marker gene copies); dsr amount of sulphur-reducing microorganisms (detected as dsr marker gene copies)

12 h long photoperiod, light intensity 370  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , temperature (day/night) 20/12 °C, relative air humidity (day/night) 45/70%. The number of plants was reduced to 13 in each pot after 14 days. The barley seedlings were grown for 12 weeks and then harvested at ground level for biomass analysis. From each pot, i.e., four per every type of tested treatment, the mixed soil sample (weighing 100 g) was taken with T-style handle soil sampler probe and prepared for analyses.

Then, the pots with remaining soil and barley roots were sown with five maize (*Zea mays* L) seeds per pot, which were reduced to 2 plants after 14 days. Then, the experiment was carried out under the same growth conditions for more 12 weeks, i.e., 24 weeks in total, following same crop harvesting and soil sampling protocols as described above.

#### Plant biomass and soil properties determination

After barley and maize seedlings were cut at the ground level, they were washed and dried at 60 °C to constant weight. The dry above ground biomass (AGB) was determined gravimetrically using the analytical scales.

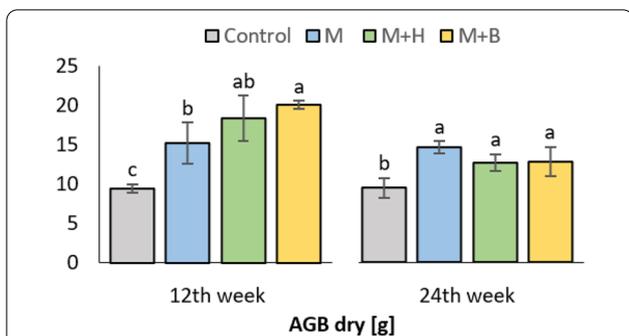
A mixed soil sample per pot taken after the harvesting of plants was homogenized by sieving through a 2 mm mesh. Air-dried samples were used for soil pH

(CaCl<sub>2</sub>) determination [30], total soil carbon (TC), nitrogen (TN), and sulphur (S) content determination with the Vario Macro Cube (Elementar Analysensysteme GmbH, Langensfeld, Germany). The C:N ratio was calculated from TC and TN values. The freeze-dried samples were prepared for the enzyme activity assays according to [31], namely:  $\beta$ -glucosidase (GLU), arylsulfatase (ARS), phosphatase (Phos), N-acetyl- $\beta$ -D-glucosaminidase (NAG), and urease (Ure).

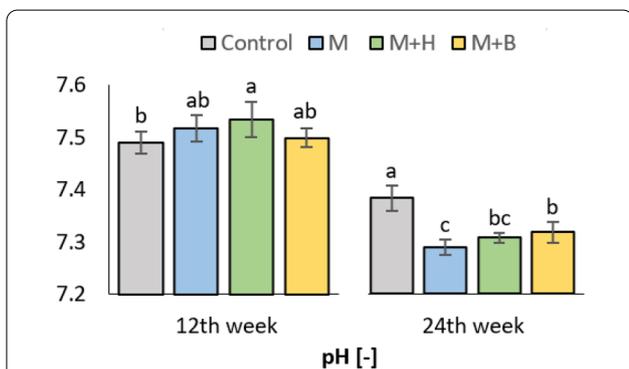
#### Statistical analyses

Data obtained from the performed measurements were statistically analyzed using the methods of multivariate analysis of variance (MANOVA), principal component analysis (PCA), one-way analysis of variance (ANOVA), Tukey HSD post-hoc test (at significance level  $p = 0.05$ ), and Pearson correlation analysis (Program R, version 3.6.1—[32]).

The Rohlf's PCA was used to evaluate the mutual dependence among the properties and their values in individual compared variants of amended soil. The results of Pearson's correlation analysis were mentioned when value of the correlation coefficient  $r$  was:  $0.5 < r < 0.7$  (moderate correlation) and  $0.7 < r < 0.9$  (high correlation) [33].



**Fig. 1** Dry aboveground biomass of barley grown in 12 weeks and maize grown in the following 12 weeks of cultivation



**Fig. 2** Soil pH after 12 weeks and 24 weeks of experiment

**Results**

**Plant traits under applied amendments**

After 12 weeks of growth, the significantly highest dry AGB was detected in the M+B variant. However, the M+H variant showed no statistical difference as compared to M variant, which was also significantly higher as compared to the control (Fig. 1). In the later phase of growth (after following 12 weeks), the control reached again significantly lower dry AGB compared to the other variants. However, the M, M+H and M+B did not

significantly differ in their positive effect on plant biomass (Fig. 1).

Average values ( $n=4$ ) are displayed, error bars are standard error of mean. The different letters of variant values indicate a statistical difference between them at the level  $p \leq 0.05$ .

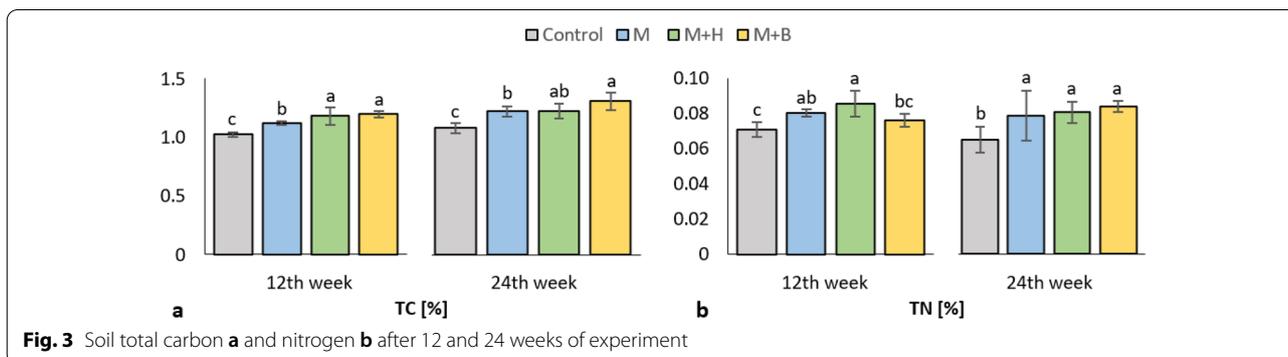
**Soil properties**

In the first part of the experiment, after 12 weeks of barley cultivation, there was no significant difference in pH values among the control, M and M+B variant. However, M+H variant showed significantly increased pH value as compared to the control (Fig. 2). At the end of experiment (24th week), soil pH was significantly lower in all manure-based variants (M, M+H, M+B) as compared to the control (Fig. 2).

Average values ( $n=4$ ) are displayed, error bars are standard error of mean. The different letters of variant values indicate a statistical difference between them at the level  $p \leq 0.05$ .

After 12 weeks of experiment, an increase of 17%, 15% and 9% in TC content of the soil was observed in M+B, M+H, and M treatments than control, respectively (Fig. 3a). The traits determined in the half (12th week) of cultivation correlated significantly ( $p \leq 0.001$ ) and highly as well: TC with TN, dry AGB and Ure activity ( $r$  was 0.74, 0.74, 0.65, respectively).

Soil TC was also significantly increased in all manure-amended variants after 24 weeks of experiment, compared to the control (Fig. 3a). Moreover, significant difference was still apparent between M+B and M variant. The highest total carbon was derived by biochar amendment and TC content showed significant high correlation with TN ( $p \leq 0.001$ ,  $r=0.74$ ) and dry AGB ( $p \leq 0.01$ ,  $r=0.73$ ). A significantly differing TC content (9.10%, 12.23%, 21.01%—Table 2) in the applied manures M, M+H, M+B, respectively, was predisposition for the long-time effect of manures modified with carbon-rich amendments on the soil TC enrichment:



**Fig. 3** Soil total carbon **a** and nitrogen **b** after 12 and 24 weeks of experiment

the increase by 13%, 14%, 21% was revealed in the soil variants M, M+H, M+B, respectively, compared to the control.

Average values ( $n=4$ ) are displayed, error bars are standard error of mean. The different letters of variant values indicate a statistical difference between them at the level  $p \leq 0.05$ .

After 12 weeks, only M and M+H contained significantly higher amount of TN compared to the control, whereas TN in M+B was significantly lower in comparison to M+H. After 24 weeks, soil TN was significantly increased in all manure-amended variants, compared to the control (Fig. 3b). However, no significant differences were detected among M, M+H, and M+B.

Results indicated that in the first part of experiment (12th week) the biochar (in M+B) had the most prominent effect on carbon content in soil, as evidenced by the significantly highest C:N ratio from all variants. Whereas at the end of the experiment (24th week), the C:N value

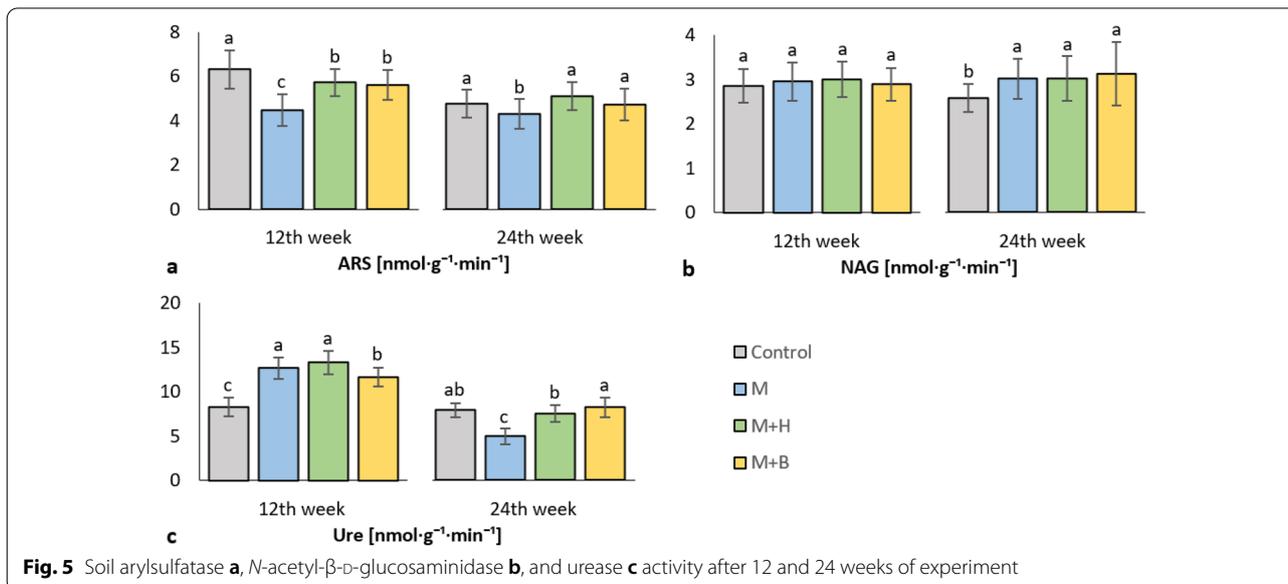
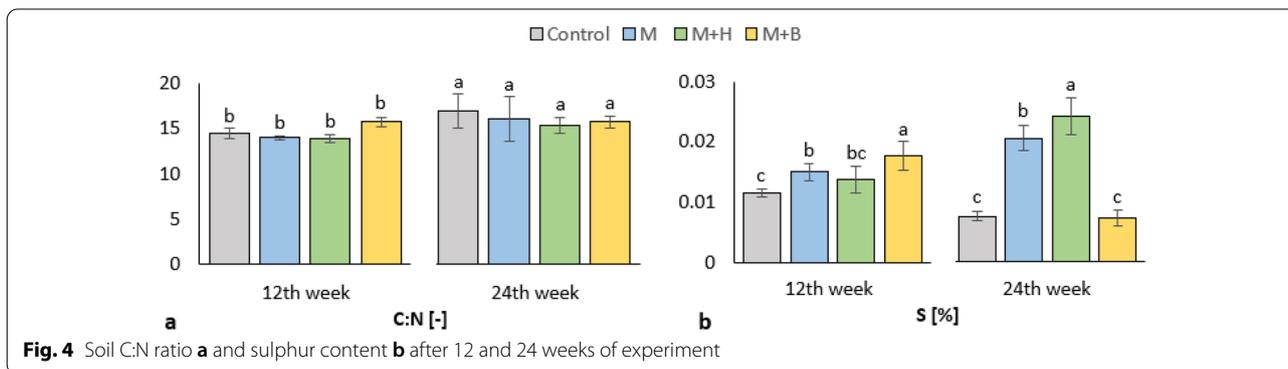
in M+B decreased and showed no significant difference among the other variants (Fig. 4a).

Average values ( $n=4$ ) are displayed, error bars are standard error of mean. The different letters of variant values indicate a statistical difference between them at the level  $p \leq 0.05$ .

Soil S values after 12 weeks of experiment were significantly increased, compared to the control, in M and M+B (Fig. 4b), as response to the putatively higher content of S in manure and biochar compared to Humac. However, the M and M+H variants showed significantly higher soil S content as compared to the control and M+B variant at the end of experiment (Fig. 4b).

### Soil enzyme activities

The arylsulfatase (ARS) values, determined after first 12 weeks of barley cultivation, were significantly lower in all manure-amended variants (M, M+H, M+B) as compared to the control (Fig. 5a). Moreover, amendment



with manure (M) mediated significantly higher suppression of ARS activity in comparison to the enriched manures M+H and M+B, because its S content (0.79%) was 2.1-fold and 2.7-fold higher compared to the S content of M+H (0.37%) and M+B (0.33%)—Table 2. Such presumed suppressive effect of increased S availability on the ARS activity was still apparent at the end of experiment and lowered ARS activity in M variant as compared to the control, as well as M+H and M+B (Fig. 5a).

Average values ( $n=4$ ) are displayed, error bars are standard error of mean. The different letters of variant values indicate a statistical difference between them at the level  $p \leq 0.05$ .

In the early phase of experiment, NAG activity showed no significant difference between all tested variants (Fig. 5b). Whereas at the end of experiment, NAG activity was significantly increased in all manure-amended variants (M, M+H, M+B) in comparison to the control (Fig. 5b).

In shorter interval (12th week), Ure values were significantly increased after the application of manure-based amendment in variants M, M+H, M+B as compared to the control (Fig. 5c). The Ure values of soil variants M and M+H were observed significantly higher compared to the M+B variant, which relation correspond to the values of TN in the respective manure types, which were (in average) 2.48%, 2.49%, 1.99% in manures M, M+H, and M+B, respectively, Table 2. The end-value of Ure was significantly the lowest in M variant and M+H variant showed significant decrease in comparison to M+B (Fig. 5c).

The early part of experiment revealed the significantly increased GLU activity in both variants amended with enriched manures (M+H, M+B) as compared to the control and M (Fig. 6a). The coupled effect of soil carbon sequestration and GLU activity mitigation was apparent from the comparison of average values of GLU in the half-time (12th week) and the end (24th week) of experiment, which in time course decreased to 97%, 99%, 72%, and 80% in variants control, M, M+H, and M+B,

respectively. On the contrary to the results from early cultivation, the end-values of GLU activity did not differ from each other between the control, M and M+H (Fig. 6a).

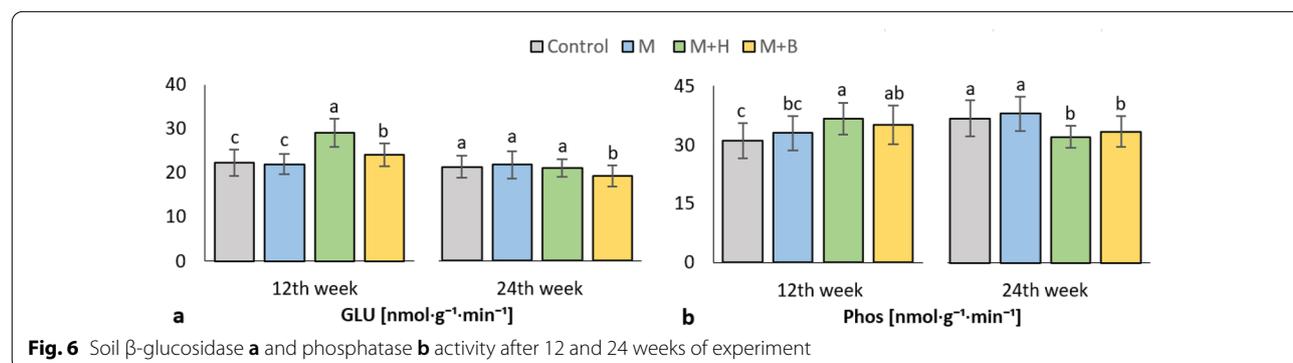
The soil Phos activity was significantly increased in M+H and M+B variants as compared to the control in the half of experiment (12th week), and the M+H variant reached significantly the highest Phos value (Fig. 6b). At the end of experiment, we observed significantly higher Phos activity in the control and M variants in comparison to M+H and M+B (Fig. 6b).

Average values ( $n=4$ ) are displayed, error bars are standard error of mean. The different letters of variant values indicate a statistical difference between them at the level  $p \leq 0.05$ .

## Discussion

### Plant traits under applied amendments

Dry aboveground biomass of cultivated barley (dry AGB) responded significantly to the changes in the pH value, nutrient contents, and enzyme activities during the early phase of cultivation experiment, which we experienced and described above. Statistically significant increase in the dry AGB values of the variants M+H and M+B (in comparison to the control, and M variant) proved the beneficial effects of soil treatment with Humac- or biochar-enriched manure on plant biomass yield in the short-term growth experiment (12 weeks). We presumed this effect from the increased TC, S, GLU, and Phos compared to the control and M soil. Positive effect of manure and biochar co-application on plant biomass and crop yield has been reported previously [34, 35], however, only under stress or other growth-limiting conditions as nutrient-poor sandy soil. Nevertheless, the benefit of M+H and M+B amendments was no longer detected at the end of experiment (24 weeks). No significant difference was found among the variants M, M+H and M+B in their positive effect on plant (maize) biomass (Fig. 1). This finding was in contrast with the difference in soil



traits which still were significantly different among M, M+H and M+B (e.g., ARS, Ure, Phos, S; pH, TC, GLU for M+B only). We ascribe the mitigation of positive effect of amendments Humac and biochar to their putative impact on soil organic matter (SOM) accumulation and nutrient sequestration via stabilization. This effect is well known, and we assume its role in the previously referred weak benefit of biochar or Humac co-application with fertilizers for plant biomass yield [36–38], despite a proved improvement of several soil quality indicators and properties (Fig. 3).

### Soil properties

In the early cultivation phase (after 12 weeks), only M+H variant showed significantly increased pH value in comparison to the control (Fig. 2). This immediate impact of the added manure to M+H soil variant is explainable with respect to the pH average values of the amended manures, which were 9.04 (M), 9.25 (M+H), and 8.71 (M+B), respectively (Table 2). Despite lower pH of M+B manure (as compared to the manure M), the long-time effect of biochar-enriched manure is pH stabilizing, putatively due to the sorption and neutralizing properties of biochar [39]. Neutralizing properties could be ascribed to the biochar-mediated sequestration of basic (alkaline) ions (e.g.,  $\text{Ca}^{2+}$ ) or immobilization of  $\text{NH}_4^+$ . If not immobilized on biochar surface, reduced ammonium, which remained intact in M manure, could release protons during nitrification [40] and plant uptake, and may possibly cause acidification. In the 24th week of the experiment, all manure-based variants (M, M+H, M+B) exerted significantly decreased soil pH in comparison to the control (Fig. 2 Soil pH after 12 weeks and 24 weeks of experiment). Moreover, the significant drop in pH of the M variant was revealed, in comparison to the M+B variant. The SOM of manure-amended variant was expected to acidify the soil as compared to the control [41]. The SOM was assumed being associated with higher TC content and plant nutrition as well, which is documented by the moderate to high significant ( $p \leq 0.001$ ) negative correlation of pH with TC ( $r = -0.61$ ) and dry AGB ( $r = -0.83$ ).

The significant increase in soil TC was evidenced for all manure-based variants after 12 weeks (M+H and M+B significantly higher than M) and after 24 weeks (M+B significantly higher compared to M) of the experiment duration. Regarding this finding, it was evidenced that the biochar represented a more recalcitrant form of carbon source, in comparison to Humac amendment. The increase in TC values between 12 and 24th week of experiment was 10%, suggesting carbon sequestration ability of applied amendments. Similar ability of biochar

( $24 \text{ t ha}^{-1}$ ) to improve SOC stocks and improve C stability was reported by Li et al. [42].

Soil TN was significantly increased in M and M+H variants in comparison to the control in the 12th week as well as 24th week (Fig. 3b), when TN value of M+B variant was significantly higher than TN value of control too. These findings agreed with the amount of amended nitrogen, which was 1.25-fold higher in manure M+H than in manure M+B (Table 2). The comparison of soil TN values in 12th and 24th week of experiment showed that TN of the control, M and M+H decreased by 8%, 2%, and 6% in average, respectively, whereas M+B exerted +10% increase in TN over time. These data confirmed the assumed positive impact of biochar enrichment on the soil nitrogen sequestration due to the reduction in leaching, as reported by Ullah et al. [43]. Content of TN was presumably significantly related to the organic nitrogen sources in soil, which is supported by the significant ( $p \leq 0.001$ ) moderate positive correlation with Ure activity (values of 12th week,  $r = 0.56$ ) and the results of PCA biplot analysis (Fig. 7): agonistic relation with NAG and Ure (12th week) and with NAG only (24th week).

C:N ratio determination revealed that M+B amendment conferred the significantly highest C:N ratio from all soil variants in the halfway through the experiment. But in the end, the C:N value in M+B soil variant decreased. We ascribe that the biochar provided a portion of utilizable carbon and at the same time stabilized the soil nitrogen from losses by either leaching or volatilization [44].

The S content in M+B decreased in average by 58% between 12 and 24th week of experiment, which ascribed that S was removed during the mineralization process. If we anticipate nitrogen-enrichment of biochar in M+B, assuming this from significantly highest content of ammonium in the manure M+B (Table 2), we can explain the observed results by processes similar to those reported by Bimer [45]. The work by Bimer referred to nitrogen-enriched carbons showed potential to remove hydrogen sulfide or sulfur dioxide from soil. Contrasting to the biochar, the effect of Humac on the S content in variant M+H may be related to the referred complexing of ARS and humic acids [46], which is a prerequisite for increased S transformation into soil sulphate. The in-soil-left barley roots might represent a source biomass, which decomposition released the organic S source compounds.

### Soil enzyme activities

The arylsulfatase (ARS) is an enzyme involved in mineralization of organosulphates and thus related to the amount and availability of mineral S forms in soil. The manure represents an S-rich matter (mostly in form of

sulphates), therefore, manure M (due to its high S content, likely inorganic, Table 2) mediated significantly lowest ARS activity in comparison to the manures M+H and M+B. Impact of availability of mineral S on the ARS activity was still detected at 24th week and led to the significantly lowest ARS in M variant. In contrast, ARS activity of manures M+H and M+B in 24th week was comparable to the control, indicating the prolonged stimulation. Bimer [45] observed that nitrogen-rich charred matter stimulated the removal of hydrogen sulfide and sulfur dioxide from soil, similarly, enhanced microbial enzyme activity could be mediated by biochar in manure as well. Some microbes possessing the arylsulfatase activity are belonging to the group of nitrifying bacteria, which may concurrently express a nitrogen-mineralizing activity as well. For example, Zak et al. [47] reported the role of *Comamonadaceae* in the mineralization of carbon-bound sulphur in the rhizosphere. Such a presumed relation between soil nitrogen-associated microbial activity and ARS-mediated mineralization could be corroborated by weak correlation of ARS with Ure ( $p \leq 0.001$ ,  $r = 0.34$ ) (Fig. 8).

N-acetyl- $\beta$ -D-glucosaminidase (NAG) is enzyme degrading chitin (nitrogenous polysaccharide), one of “building blocks” of fungal cell wall. Therefore, it is an indicator of fungal biomass in soil and determinant of nitrogen mineralization. Because the effect of manure amendment on the NAG activity was not significant in the first half of experiment (12th week), we presume that manure stimulated soil fungal biomass in soil, as previously referred [48], but this effect was gradual and long-term. The higher chitin content and degradation putatively contributed to nitrogen mineralization and sequestration, which may be ascribed from the previously mentioned agonistic relation between NAG and TN (PCA biplot—Fig. 7).

The main indicator of nitrogen mineralization in soil in our experiment was Ure an enzyme occurring commonly in all types of soil organisms (Fig. 5c). The Ure values were significantly increased in shorter interval in all manure-amended variants compared to the control: M and M+H were observed significantly higher compared to the M+B variant. However, the end-value of Ure was significantly the lowest in M variant, M+H variant revealed significantly lower Ure compared to M+B. We ascribed that the manure-derived nitrogen sources increased Ure activity similarly to referred [49], on the contrary to the NAG activity was this Ure effect short-term and vanished at the end of experiment (24th week). We assumed that biochar-mediated nitrogen sequestration and stabilization in soil M+B resulted in slightly lower rate of Ure-associated degradation of hydrolysable nitrogenous substrates (as compared to M and M+H).

This putatively resulted in long-term stable Ure activity at moderate rate, similarly to less induced Ure in the control soil, from the reason of lower access of degradable N-substrates. Such a variable biochar-derived Ure response during growth period of tested crop was already reported [50], the activity showed first increase, then decrease, and in the heading stage Ure was the highest.

Significantly higher GLU activity (compared to the control and M) of variants M+H and M+B in the first half of the experiment was putatively related to total carbon content in soil, documented by GLU and TC mutual correlation ( $r = 0.43$ ). This finding agreed also with the referred impact of manure on the increase of GLU activity [51]. We presume a stimulating effect of higher carbon access (12.23%, 21.01% TC in manures M+H, M+B, Table 2) in amended manure on general carbon-catabolic activity of soil biota, because this beneficial effect of the respective amendments (Humac, biochar) is known [38, 52, 53]. Nevertheless, the positive effect of Humac in manure (M+H) was significantly higher than the effect of manure with biochar in M+B variant, we assume a partial negative priming effect of biochar on GLU activity, similarly as it was referred [36]. The GLU activity of final samples did not show differences in the control, M and M+H (Fig. 6a). However, the M+B variant showed significantly the lowest GLU activity, which finding we explained as result of increased carbon accumulation in the M+B soil, the variant of amendment with presumably the lowest ratio between oxidizable and recalcitrant carbon. Decreased activity of GLU joint with increased C accumulation in the biochar-amended soil was referred by Zhang et al. [54] as well.

The soil Phos is enzyme involved in mineralization of phosphorus in soil [55]. Phosphorus (P) is also an important component of manure [56]. The M+H and M+B variants exerted the significantly increased soil Phos activity was in comparison to the control in the 12th week, M+H variant was significantly higher compared to the M variant. Again, we explained this with enrichment of manure with Humac and biochar (P-containing matters) provided higher phosphorus access and increased demand of soil microbiota and plants (with dry biomass higher compared to M variant) in M+H and M+B variants for available form of phosphorus. The available phosphorus in manure variants (M, M+H, M+B) decreased (4.2, 3.6, 3.0 g·kg<sup>-1</sup>, Table 2) with the ascending content of TC, and the trend of differences in Phos activities among soil variants was similar to GLU. For Phos behavior in M+B amended soil, we assumed that a negative priming effect of biochar hindered the increase of its activity in comparison to the M variant, similarly referred by Foster et al. [36]. We found the observed positive effect of Humac on Phos activity in line

with results reported by Bastida et al. [57]. The significant ( $p \leq 0.001$ ) correlation between Phos and GLU ( $r = 0.45$ ) supported our outcomes as well. Therefore, the coupling of Phos and GLU was also significant at the end of experiment, when the control and M variant again showed significantly higher Phos activity as compared to M+H and M+B variants (Fig. 6b). The significant correlation between Phos and GLU ( $p \leq 0.001$ ,  $r = 0.38$ ) supported presumption of lower availability of organic phosphorus for dephosphorylation by Phos in those variants, which concurrently showed low  $\beta$ -glucosidase activity. A similar decrease in significance of positive effect of humic acids amendment on soil Phos activity in long-term cultivation was referred [58], as well as negative correlation between total organic carbon and Phos [59] in soil amended with composted matter.

### Conclusions

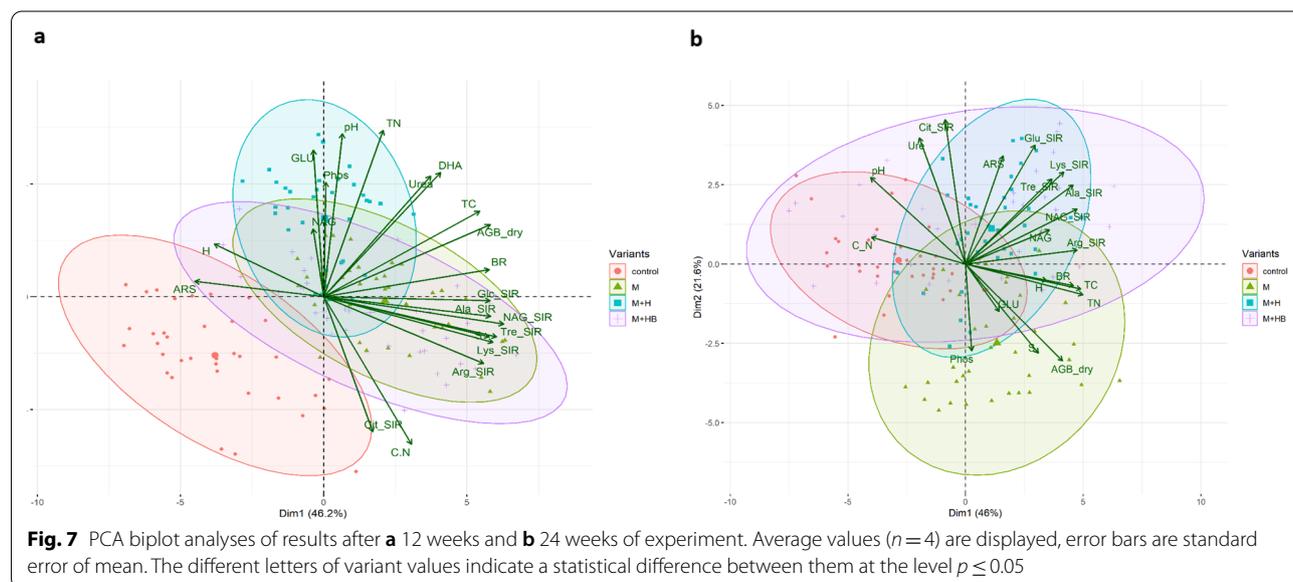
This study concluded that the manure variants differed in their impact on observed soil and plant properties owing to their composition and the interval of influence after single application. The most significant effect on the soil properties including pH, TC, TN, and  $\beta$ -glucosidase, phosphatase, urease activities was observed for the M+H variant in the half-time of the experiment (12 weeks of barley cultivation). While the M+B variant showed the significantly highest C:N ratio, soil sulphur content, and dry aboveground biomass of barley after 12 weeks of barley cultivation. It was thus concluded that the enrichment of fresh manure with carbon-rich materials prior to fermentation modified the end products into

the amendments which once applied to the soil conferred the beneficial properties. The short-termed benefit was expressed as higher nutrient content, activity of enzymes involved in the transformation of macro-elements, and higher fertilizing potential for increasing crop (barley) yield.

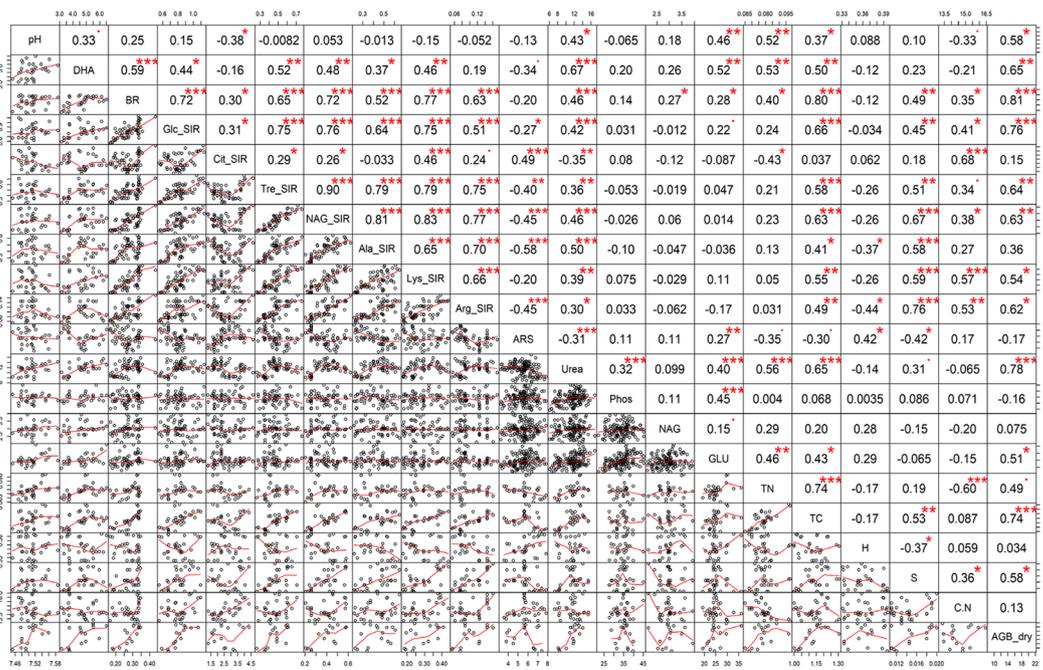
However, these primary positive impacts of M+H and M+B manures were reduced and mitigated after longer period of influence under maize cultivation, i.e., at the end of experiment (24 weeks). Compared to the control, the M+B variants showed the most significant positive effect on TC, TN, urease, as well as the lowest pH, whereas in the M+H variants the highest values of sulphur content and arylsulfatase. Prolonged effects of single-applied tested manure-based amendments declined in magnitude of alteration. Possibly, this observed weakening of beneficial effects could be prevented by shortened period of manure maturation which could preserve a higher amount of less degraded labile organic matter. As this novel approach in one-time application of co-composted carbon-rich amendment in pot soil differs from conventional practice, where manure is applied periodically, we suggest further in depth studies are required to optimize and compare the rate of organic amendments applied for crop production under shorter and longer periods of time.

### Appendix

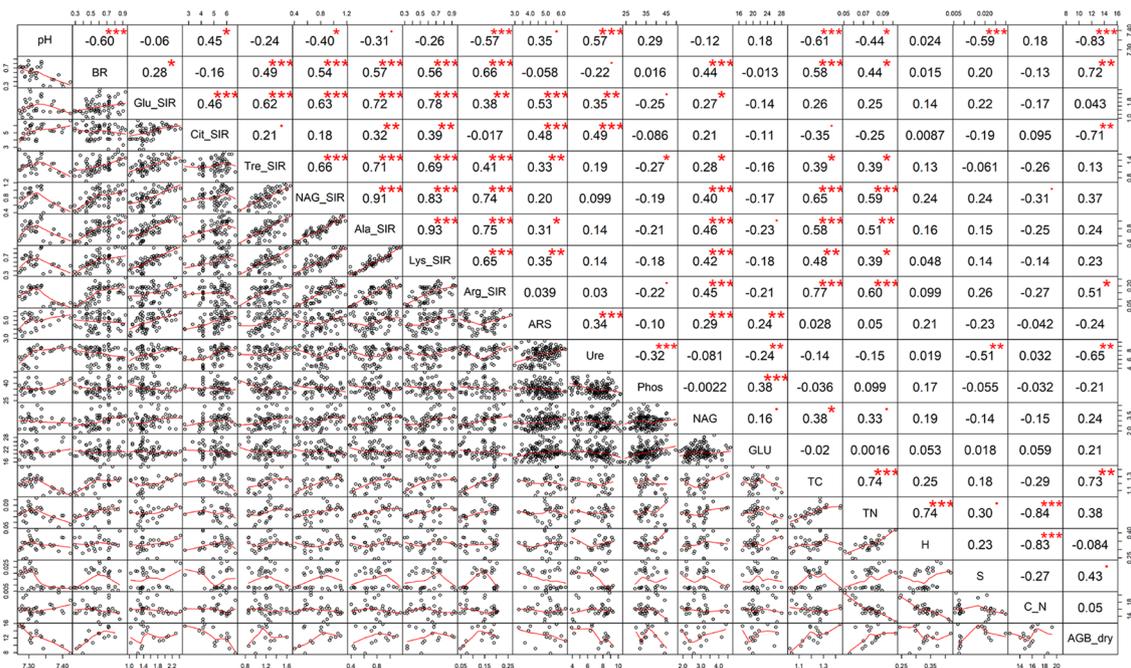
See Figs. 7 and 8



**a**



**b**



**Fig. 8** Pearson's correlation matrix of results after **a** 12 weeks and **b** 24 weeks of experiment. The stars indicate a level of significance in statistical difference between the variables: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$

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**Author contributions**

Conceptualization, TH, JH and MB; methodology, TH, JH and MB; software, TH, TB; validation, MB, MR, OM, AK and IJ; formal analysis, OL, TB; investigation, JH, AM and MB; resources, OL, OM, and AK; data curation, TH, MR and OL; writing—original draft preparation, JH, TH; writing—review and editing, TH, JH, AM, IJ and MB; visualization, TH and AK; supervision, MR, MB; project administration, MB, AK; funding acquisition, MB, AK. All authors read and approved the final manuscript.

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**Availability of data and materials**

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations****Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

<sup>1</sup>Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, 61300 Brno, Czech Republic. <sup>2</sup>Agrovyzkum Rapotin, Ltd., Vyzkumniku 267, 788 13 Rapotin, Czech Republic. <sup>3</sup>Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic. <sup>4</sup>Institute for Environmental Studies, Faculty of Science, Charles University in Prague, Benatska 2, 12800 Praha, Czech Republic. <sup>5</sup>Agricultural Research, Ltd., Zahradni 400/1, 664 41 Troubsko, Czech Republic. <sup>6</sup>Institute of Environmental Engineering, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland. <sup>7</sup>Department of Agronomy, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, 7 Prof. S. Kaliskiego St., 85-796 Bydgoszcz, Poland.

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