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ORIGINAL RESEARCH

Effect of biochar, biocompost and manure on the growth and productivity of alfalfa (*Medicago sativa L.*): Field and pots study

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Abstract

Purpose Biochar is a carbon-rich coproduct resulting from pyrolyzing biomass. Positive effects on productivity, soil stability, carbon sequestration, soil fertility have been validated by several studies. The aim of the present study is to compare the effect of different rates of biochar "BC TD" (produced from the pyrolysis of seeds of date '**D**' and "Tomato residue" '**T**') on the productivity of alfalfa in pots and in the field in comparison with manure, nitrogen fertilizer and biocompost (CP).

Method In order to carry out this comparison, alfalfa was cultivated in the same bases and under the same climatic conditions, and the various physiological, growth and productivity parameters were continuously monitored throughout the period of the experiment.

Results The manure doses resulted in better productivity throughout the test period compared to all the treatments tested. For biochar, alfalfa germination results were widely different between field and pots with low germination rate in high doses of biochar which subsequently affected productivity.

Conclusion Although the application of high doses of biochar and biocompost decreases productivity and limits all growth parameters but positive results on productivity were noted with the 3% BC treatment in the field, which requires monitoring this dose for prolonged periods in order to properly determine its long-term effects on the various physiological parameters, growth and productivity of alfalfa.

Keywords Biochar, Bio-compost, Manure, Alfalfa

Introduction

The alfalfa (*Medicago sativa L.*) is the most important forage crops in Morocco (Rita et al. 2017; Villax 1963). It is usually harvested several times a year and fed to livestock either as hay or silage form (El Moussaoui et al. 2022). The alfalfa is also cultivated for seed production or for grazing. It is highly efficient and is a good source of protein available for livestock (Boller et al. 2010). In crop rotations, alfalfa improves soil structure, strengthens soil fertility and reduces pest problems for other crops (Fox et al. 2007). Generally, the application of chemical fertilizers or manure and the rotation of crops are the means available to preserve a stable productivity and quality of the alfalfa. The destruction of the soil and the microbial community, the appearance of weeds and contamination of groundwater are the major negative effects of chemical fertilizers (Kelling and Schmitt 2018; Marouane et al. 2014; Savci 2012). The problems linked to these management modes make it necessary

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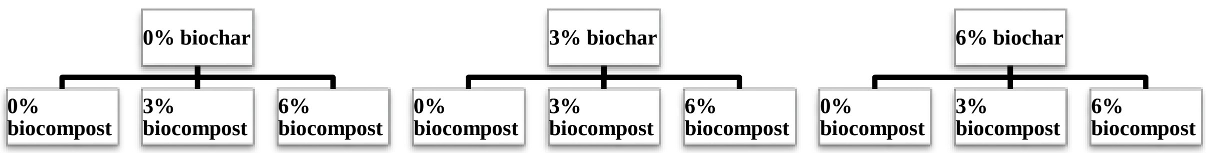
to seek other means more efficient with the minimum economic and environmental drawbacks. Biochar is the solid product of biomass pyrolysis that in recent years has shown significant economic and environmental effects; it increase productivity, preserve soil, water, sequesters carbon in the soil and improves soil fertility (Isimikalu et al. 2022; Majumder et al. 2019; Mandal et al. 2021; Yadav et al. 2019). Biochar increased pH and base saturation as well as CEC and associated nutrient retention, and available P (Major et al. 2010). It is also beneficial for water retention and soil structure by increasing soil aeration and porosity (Bouqbis et al. 2016; Rasa et al. 2018), and better nutrient retention in soil micropores (Lehmann and Joseph 2015). Biochar improves the supply of essential nutrients and thus plays a critical role in nutrient cycling (Biederman and Stanley Harpole 2013). Biochar positively influences soil enzyme activities and stimulatesseveral microbial groups, allowing improved uptake of nutrients by plants and results in significantly higher root mass (Kocsis et al. 2020). Combining biochar with compost can increase these benefits (Agegnehu et al. 2015; El Moussaoui and Bouqbis 2022). Careful selection of feedstock is crucial as it must be abundantly available, inexpensive, unavoidable, and have a low embedded impact (El Moussaoui and Bouqbis 2022; Oldfield et al. 2018). The combination of organic amendments with mineral P sources could be successfully used as a cost-effective management practice to enhance soil fertility and crop production in the arid and semi-arid regions stressed with water scarcity and natural resource constraints (Ding et al. 2020). Manure is the most well-known alfalfa amendment (Basso and Ritchie 2005; Martin et al. 2006). Several studies have shown that the application of biochar especially on nutrient-poor soils increases productivity and yield (El Moussaoui and Bouqbis 2022; Jeffery et al. 2017). However, some negative effects such as potential source of toxic substances, salinity, high content of heavy metals and the presence of polycyclic aromatic hydrocarbons (PAHs) are linked to the addition of biochar to arable land, this can decrease the productivity, yield and physiological activity of plants (Buss and Mašek 2014; Rombolà et al. 2015; Visioli et al. 2016). Our hypothesis was that the productivity of alfalfa grown in soils amended with biochar and biocompost can be comparable to that grown in soils amended with manure and chemical fertilizers. The objective of this study is to test the effect of different rates of biochar on different parameters of alfalfa productivity. This study also aims to compare the productivity results of alfalfa grown in pots and those grown in the field.

Materials and methods

Preparation of mixtures

The culture mixes were prepared from all possible combinations of doses 0, 3 and 6% between biochar and bio-compost (Scheme. 1), the soil used in this study was free from any chemical contamination either pesticide or fertilizer. Biocompost was commercial product and biochars were produced from the pyrolysis of two different organic feedstock (seeds of date '**D**' and tomato residue '**T**') at a temperature around 540 °C. These two biochars were mixed together at 50% for each. Six controls are used in this study, a negative control with just the soil and three positive controls based on cattle manure '**M**' at 0.46, 1.7 and 4.15% respectively, and two other controls based on biocompost '**CP**' at 3 and 6% relative to the soil.



Scheme. 1 Different combinations between biochar and biocompost

Characteristics of pots and field

A single type of soil "sandy loam" which was used as a base to prepare the different mixes either in the field or in pots, while the same conditions, mixes and replicates were used in the field and in the pots. Temperature and humidity were monitored throughout the five month study through **DATA LOGGER UT330B** (Fig. 1). The two tests either in the field or in the pots were applied at six repetitions in the region of Taroudant in Morocco.

Characterization of pots: The pots that were used in this study have a total capacity of two liters.

Characterization of field: The field has been ploughed twice, the first is deep at 1 m and the second is shallow at 20 cm, the holes formed in the field having a cubic shape with a 13 cm ridge.

Cultivation, Irrigation and monitoring of germination, growth and productivity

Fourteen seeds of the alfalfa (*Medicago sativa L.*) registered in the Organisation for Economic Co-operation and Development OECD seed system were grown in each replicate, irrigated with a frequency of once every three days. The number of seeds germinated and the number of leaves emerged in each repetition were monitored every two days until the twenty-ninth day. After this period, this monitoring wasno longer possible and then the length of the alfalfa was measured starting on the thirty-fifth day every seven days.

Photosynthesis was measured using an open infrared gas analysis (IRGA) (ADC BioScientific LCi-SD System Serial No.33774) just before each cut of the alfalfa after two months of sowing. The alfalfa cut was done in the month of December, February and March; the fresh weight of each repetition was recorded. A nitrogenous fertilizer of type N 33.5% was added to control 1 and 2 with doses 0.002 and 0.009% respectively. This protocol has been applied for both cultivation approaches either in the field or in the pots.

Biochar, biocompost and soil chemicals analysis

Mineral content were determined using flame emission spectrophotometer for total Ca, Mg, K and Na. Fe, Mn, Zn, and Cu content were determined by atomic absorption spectrophotometer (Lindsay and Norvell 1978), The KH_2PO_4 and $NaNO_3$ were measured using colorimetrical analyses, The total soil organic carbon content was quantified by Walkley–black method and the total nitrogen (TN) content was estimated bythe Kjeldahl method. organic matter total MOT and organic carbon OC is estimated from calcination, and phosphorus is determined by the OLSEN protocol (Olsen 1954).

Data analysis

Results were evaluated statistically using Analysis of Variance (ANOVA) performed with SigmaPlot 14.5 (Systat Inc 2020). Significance of differences among treatment groups was determined with the Tukey test. Different superscript letters represent significant differences between treatments at the $p<0.05$ level (a single common letter between two treatments means that there is no significant difference between these two treatments).

Results and discussion

Biochar, biocompost and soil chemicals analysis

Table 1 indicates that the transformation modes of biomass directly influence the chemical compositions of transformed feedstock. The biocompost produced by the composting process, and the biochar produced by pyrolysis, manure and untreated soil marked widely different contents in organic composition, minerals and trace elements.

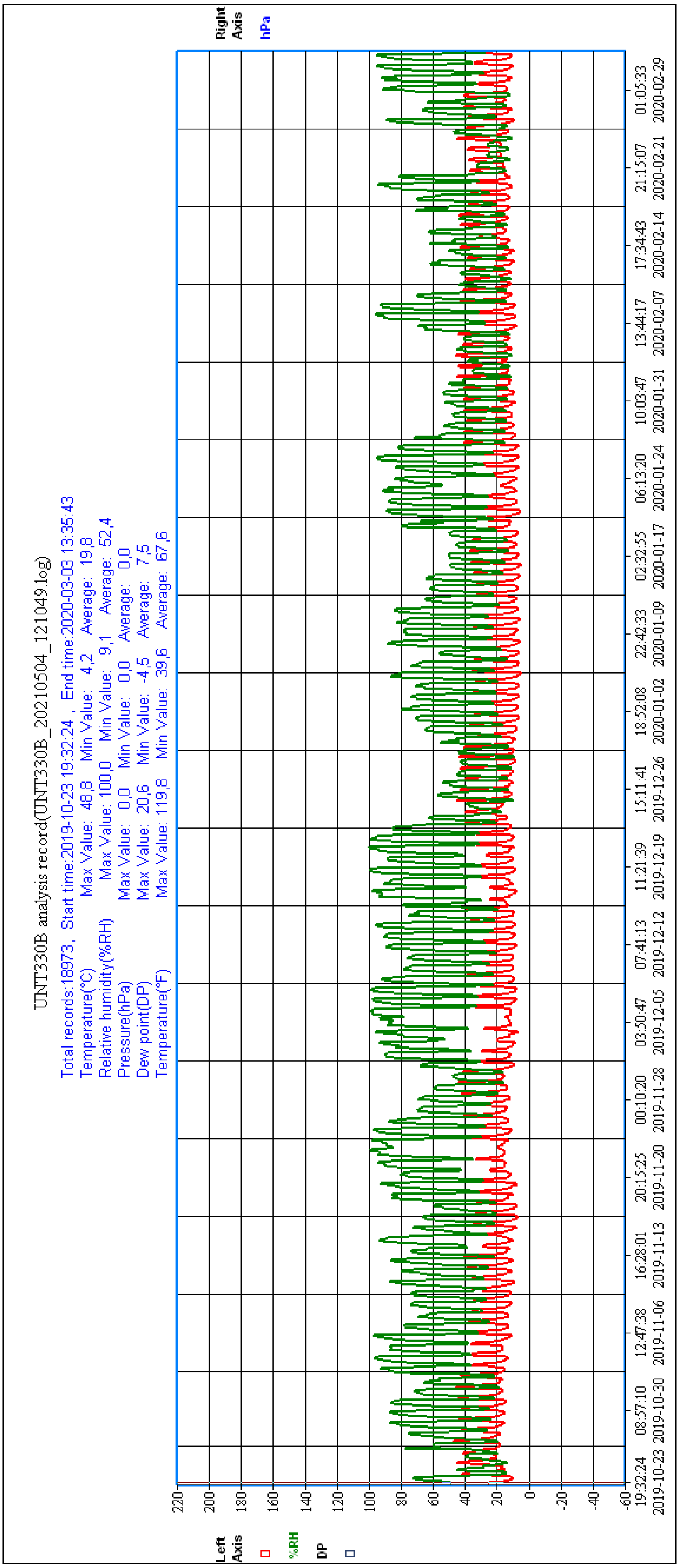


Fig. 1 Monitoring of temperature and relative humidity throughout the duration of the study « UNI-T UT330B USB Testeur Humidity/thermometer Datalogger

The soil is characterized by a low content of heavy metals that is mainly due to the choice of soil which has been fallow for more than fifteen years. However, this is accompanied by a very low content of organic matterand nitrogen.

This soil is characterized by average mineral contents with a high rate of phosphorus and magnesium reaching 0.562 and 0.715% respectively.

The biocompost has high contents of heavy metals and low contents of organic matter and NPK 0.85, 0.20 and 0.16% respectively; also a high content of Ca was observed 6.01%. The high potassium and sodium contents 8.56 and 2.69% respectively were marked in the biochar. Regarding the C/N ratio, the soil has largely high C/N ratio which mean that it must be treated with a fertilizer characterized by a high N rate. The C/N ratio for biocompost, biochar and manure 28.17, 39.2 and 22.08 respectively.

The contents of organic, nitrogen, mineral and trace element compositions were widely different between the different substrates and bases analyzed. This is mainly due to the nature of the biomasses and the transformation processes. The chemical composition of biochar is influenced mainly by temperature during the pyrolysis process and the nature of the pyrolyzed biomass.

The phosphorus content of oak biochar mayrange from 1.3 to 1.8 mg/g for temperatures between 350 and 900 °C respectively. For pine biochar, this phosphorus content in the same temperature range fluctuates between 0.47 to 0.84 mg/g (Zhang et al. 2017).

The biochar produced from urban sewage sludge in the Sydney region at 500 °C recorded high doses of heavy metals (Hossain et al. 2011). Yang et al. (2019) have shown that more than the feedstock type, the pyrolysis temperature affects the carbon content of biochar. Likewise the chemical compositions of compost are influenced by the composting conditions and by the type of organic matter (Vandecasteele et al. 2014).

The carbon and nitrogen content of the composted biomass impacts the quality of mature compost (Himanen and Hänninen 2011; Steel et al. 2012). On the other hand, the chemical composition of manure is mainly impacted by animal nutrition (Sørensen et al. 2003). The C/N ratio ofthe soil is very important to describe the degree of soil evolution (Janssen 1996). The C/N ratio for organic biomass and the process of transformation into organic fertilizer judges the quality of the organic amendment produced (Eiland et al. 2001; Guo et al. 2012; Kumar et al. 2010; Rodríguez-Vila et al. 2018).

Table 1 Biochar, Biocompost and Soil chemicals analysis

	% TOM	**% OC**	**% TN**	**C/N**	**% Pt**	**% K**	**% Na**	**% Ca**	**% Mg**	**Fe** ppm	**Mn** ppm	**Cu** ppm	**Zn** ppm
Biocompost	41.47	24.05	0.85	28.17	0.20	0.16	0.36	6.01	0.42	6079	232.5	993.8	241.4
BC TD	72.69	42.16	1.08	39.20	0.27	8.56	2.69	2.68	0.67	547	107.1	62.8	74
Manure	80.41	46.64	2.11	22.08	0.28	1	0.12	1.73	0.42	1409.6	197.2	37.5	144.9
					P_2O_5 0/00	**K_2O** 0/00	**Na_2O** 0/00	**CaO** 0/00	**MgO** 0/00				
Soil	7.09	4.11	0.004	1027.5	0.562	0.309	0.18	3.889	0.715	0.9	22.6	1.1	1.5

Monitoring of germination, growth and productivity

Germination

Fig. 2 showed that, in all doses, the germination of alfalfa in the pots was low compared to the germination in the field, except in 0.46% and 1.7% manure where they showed an insignificant increase of the pots compared to the field. The best germination percentages were marked in the field in the control 0, 3% CP, 6% CP and 6% BC 0% CP by an average around 95%. Very weak germination was detected in biochar in the pots, 3% BC 0% CP which was the best dose gave only a germination average not exceeding 40%. Although some of our treatments with biochar in field gave very interesting results, soil or biocompost alone demonstratedthe best results in terms of germination. Solaiman et al. (2012) have shown that the type of biochar and the dose play a crucial role in determining the germination rate, either positively or negatively compared to the control. In addition, germination is influenced not only by the type of biochar, but also by the ash content and the type of seeds that determine the germination rate (Reyes et al. 2015), The application of corn cob biochar stabilized the germination rate of soybean under unstressed irrigation conditions but increased this rate in those sowing under stressed irrigation conditions (Hafeez et al. 2017).

Alfalfa germination is influenced by heavy metals (Aydinalp and Marinova 2009). The substrate salinity and toxicity, especially compost-based mixtures, directly influence germination and consequently plant productivity, but these influences differ from one species to another (Luo et al. 2018).While the compost increases all the main parameters of productivity, germination and survival (Ameta et al. 2015). Generally, increasing the rate of organic matter in the soil mitigates the negative effects of salinity on germination (Masciandaro et al. 2002).

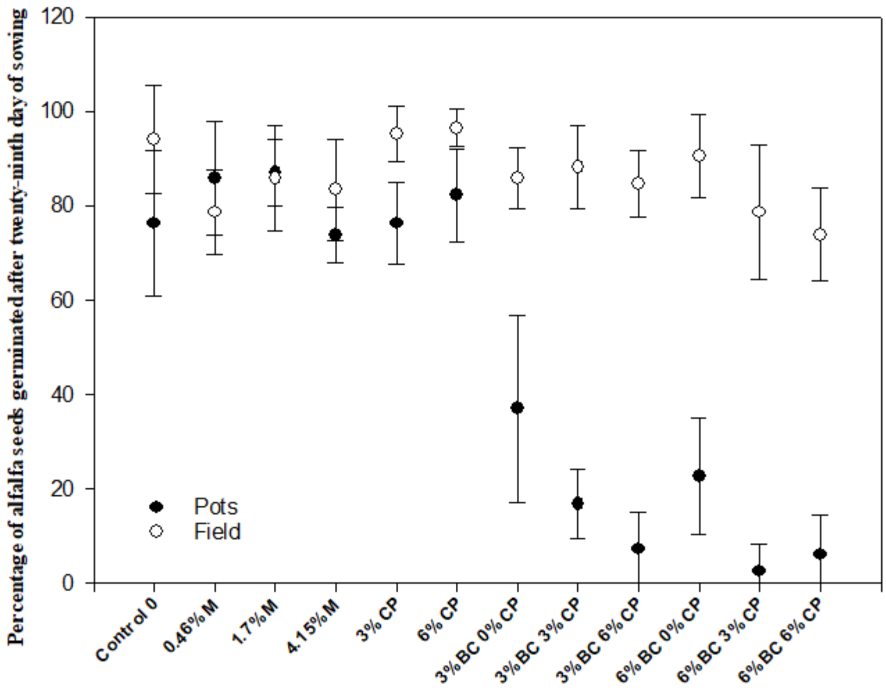


Fig. 2 Percentage of alfalfa seeds germinated between pots and field after the twenty-ninth day of sowing

BC = Biochar

CP = Bio-Compost

M = Manure

Growth

In the first view of Fig 3 and 4, it can be concluded that the statistical distribution and the effect of our treatments on the numbers of leaves is less important in the field compared to the pots, this is mainly due to the concentration of chemical elements in the closed system of pots. Besides, the roots do not have space to develop and to overcome the toxic effects of certain elements if they are present, also the fertilizers do not leach and the major part remains tight in the pots, contrary to the conditions of cultivation in the field, in the two types of culture and in all the treatments applied, the development of the number of leaves remains stable during all the twenty-nine days. This average reaches, in the pots for certain treatments, more than

200 leaves per repetition, while the number does not exceed 160 leaves by repetition in the best cases of field. The average of the number of leaves of control (0) in both types of crops remains medium compared to the treatments applied to alfalfa. The best average leaf counts were noted in manure controls and 6% bi-ocompost in both crop types. Fig. 4 indicates that the treatments 3% BC TD and 3% BC TD 3% CP in field have interesting results.

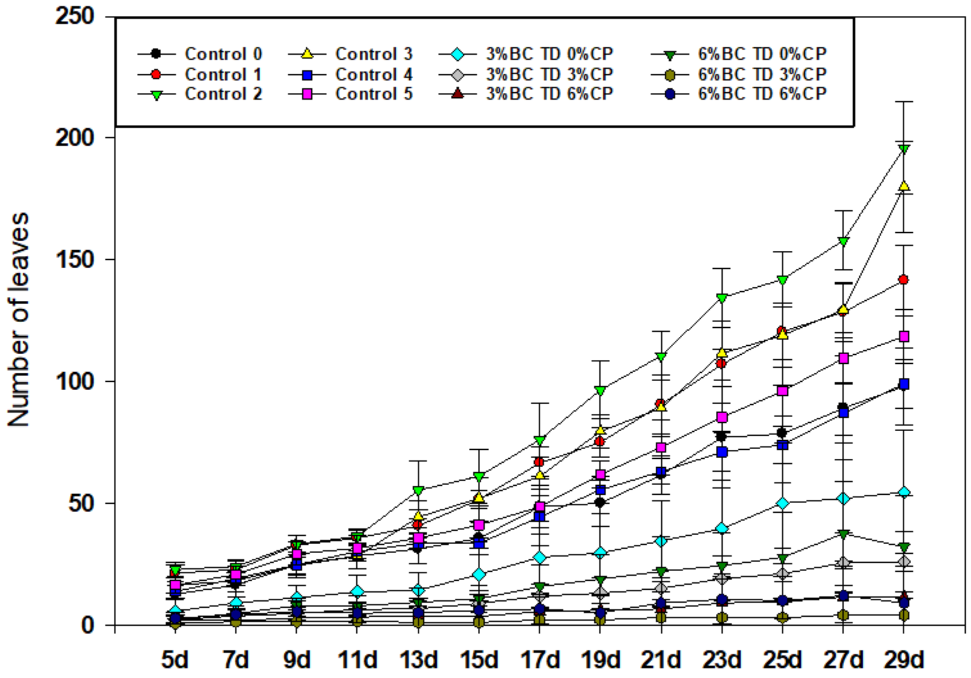


Fig. 3 Number of leaves per each treatment in pots

BC = Biochar, CP = Bio-Compost

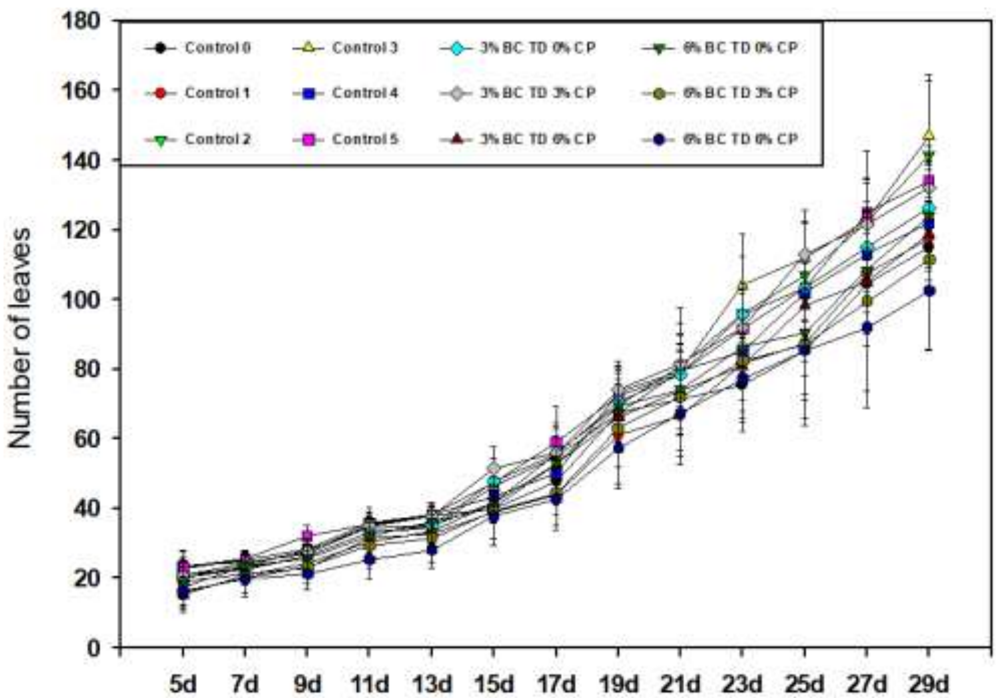


Fig. 4 Number of leaves per each treatment in field

BC = Biochar, CP = Bio-Compost

Ratio number of leaves/number of germinated seeds Fig 5 and 6 remains consistent with the results of the average number of total leaves of each repetition, which indicates that there is a strong positive correlation between these two parameters.

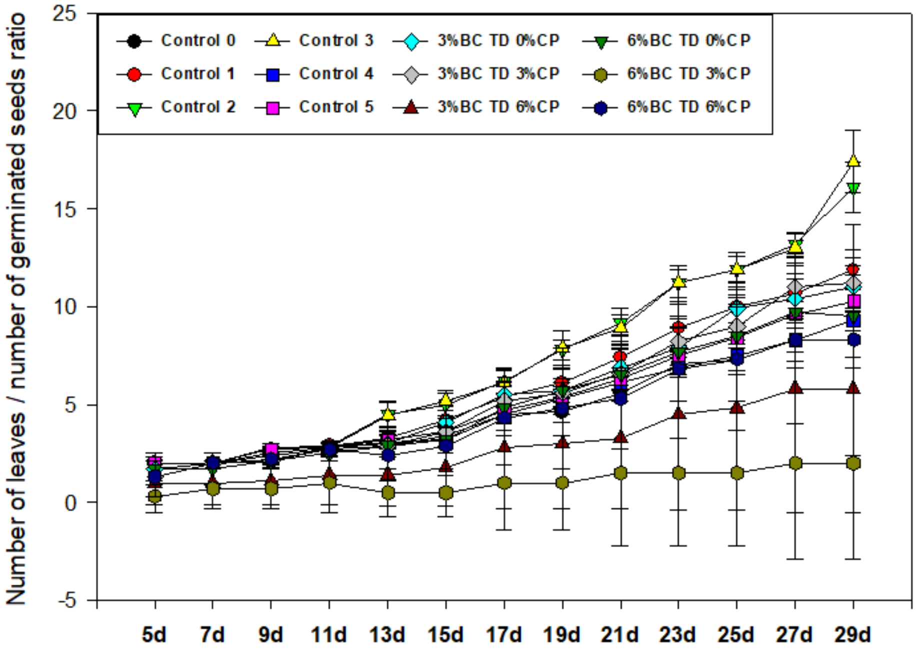


Fig. 5 Number of leaves / number of germinated seeds ratio per each treatment in pots

BC = Biochar, CP = Bio-Compost

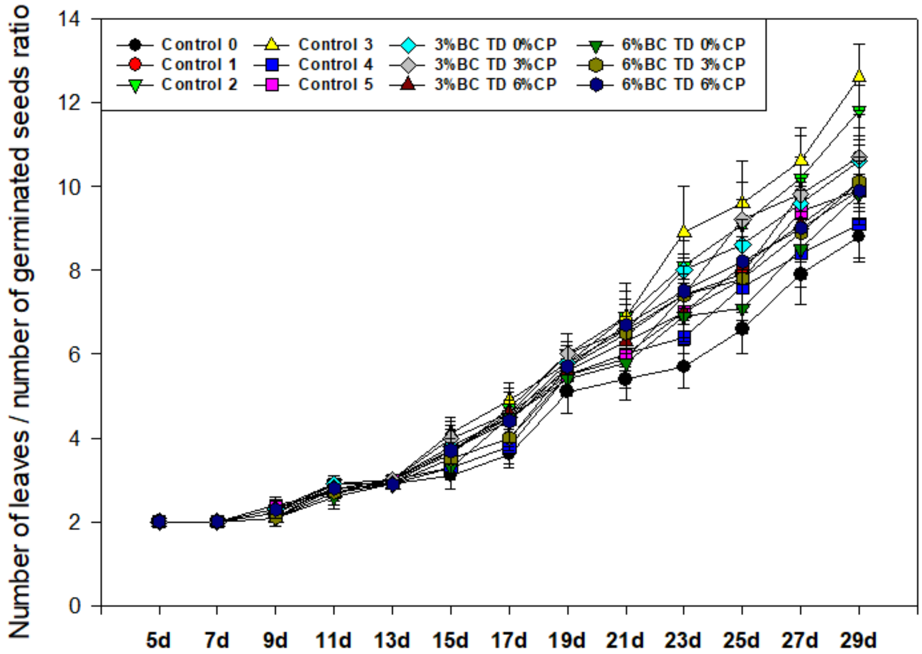


Fig. 6 Number of leaves / number of germinated seeds ratio per each treatment in field

BC = Biochar, CP = Bio-Compost

Fig 7 and 8 which depict the development of the length of alfalfa during growth and after harvest as an important means indicates the effect of treatment on the rate of maturity of alfalfa. This can decrease the time between harvests and by result increases productivity. Generally, the application of manure especially in high doses gives better and stable development of the length of the alfalfa throughout the duration of the test.

After the first harvest the 3% BC TD 0% CP treatment gave strong lengths compared to all the other treatments but after the second harvest these results decreased.

A 20% germination rate of 3% BC TD 3% CP gives better root use of the subterranean space which causes alfalfa to mature quickly compared to other treatments applied to the pots.

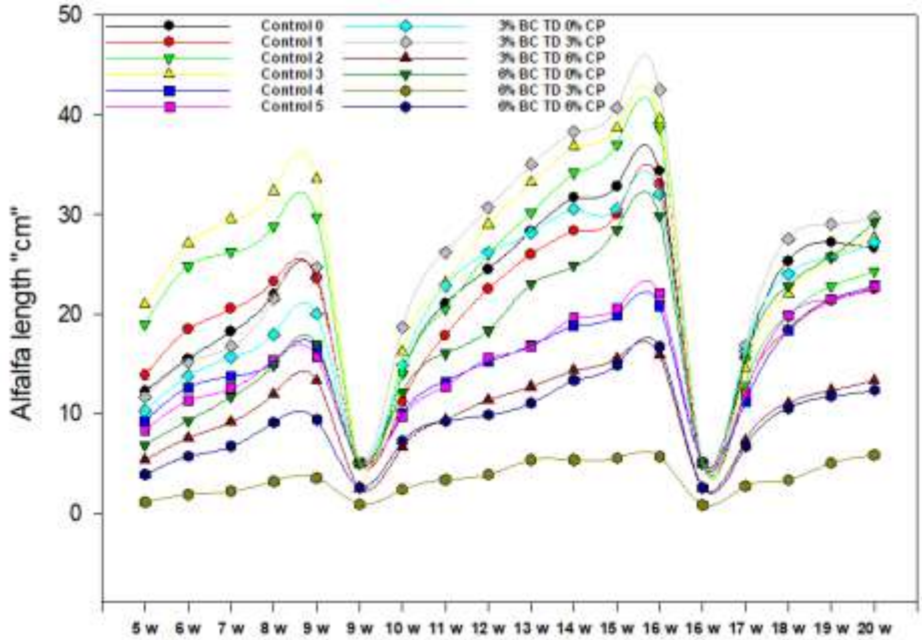


Fig. 7 Alfalfa length "cm" during the twenty weeks and three cuts in pots

BC = Biochar, CP = Bio-Compost

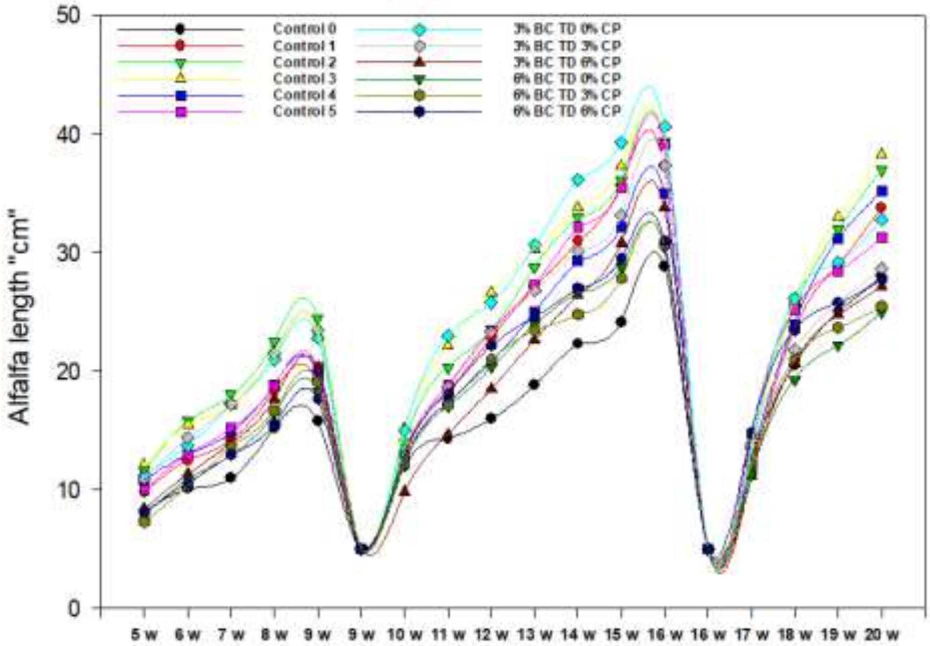


Fig. 8 Alfalfa length "cm" during the twenty weeks and three cuts in field

BC = Biochar, CP = Bio-Compost

Fresh weight was affected by the low germination rate in the pots, lower fresh weights were noted in treatments with lower germination rates, as the previous parameters. The strongest averages of fresh weight were marked in the treatments with the high doses of manure, these averages reached 20 g in some crops. In the field, the 3% BC TD treatment in second harvest resulted in weights exceeding 15 g with no statistically significant difference with the 1.7% and 4.16% manure treatments which gave better productivity. Usually high doses of biochar and biocompost decrease weight in all crops compared to controls (Fig. 9 and 10). The dry weight results were in agreement with the fresh weight results (Fig. 11 and 12).

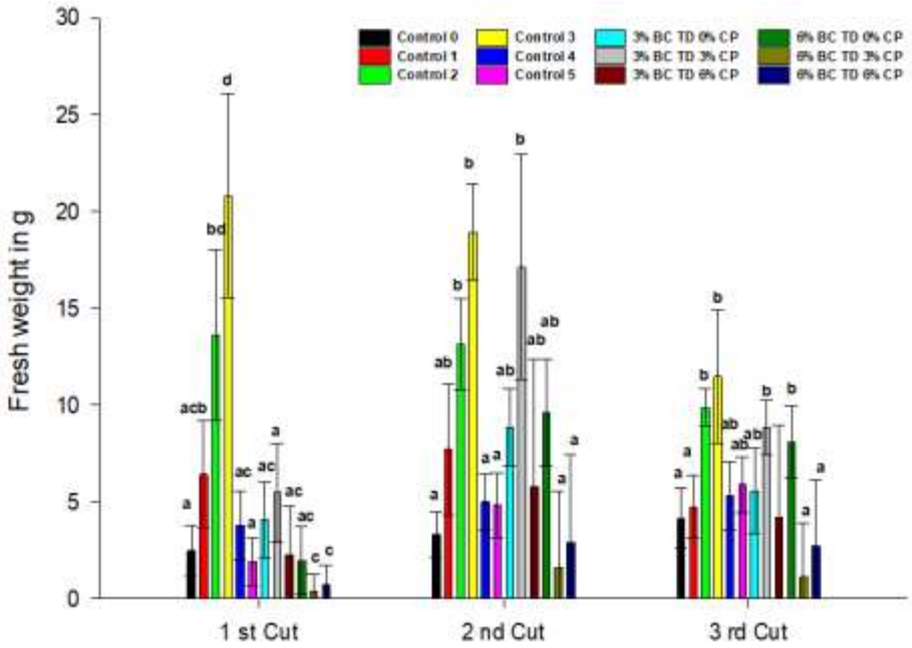


Fig. 9 Alfalfa fresh weight in pots for the first three cuts

Different superscript letters represent significant differences between treatments at the P < 0.05 level.

BC = biochar (Feedstock source), CP = Bio-Compost

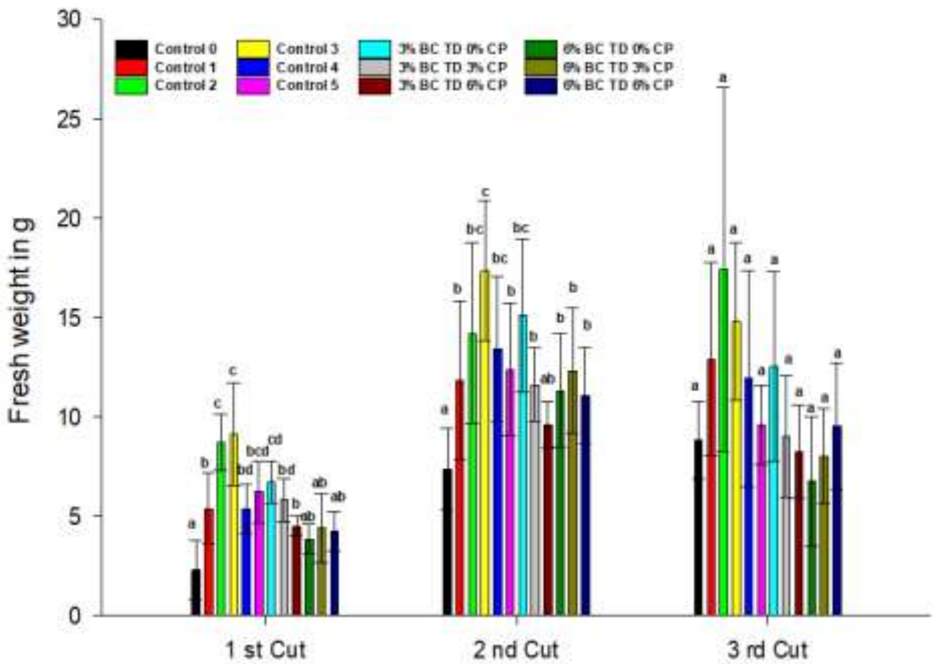


Fig. 10 Alfalfa fresh weight in field for the first three cuts

Different superscript letters represent significant differences between treatments at the P < 0.05 level.

BC = biochar (Feedstock source), CP = Bio-Compost

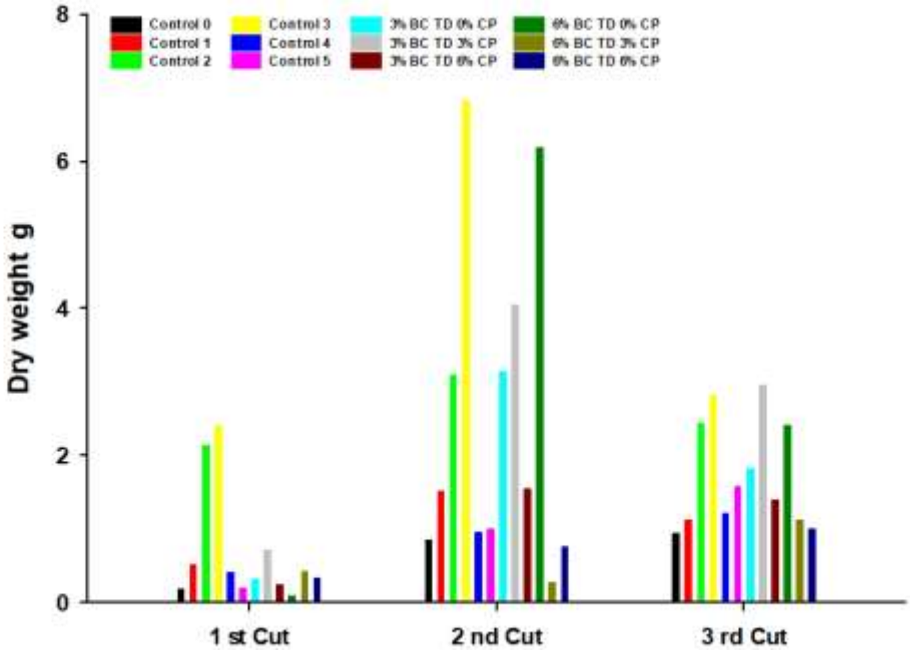


Fig. 11 Alfalfa dry weight in pots for the first three cuts

"Six repetitions" BC = biochar (Feedstock source); CP = Bio-Compost

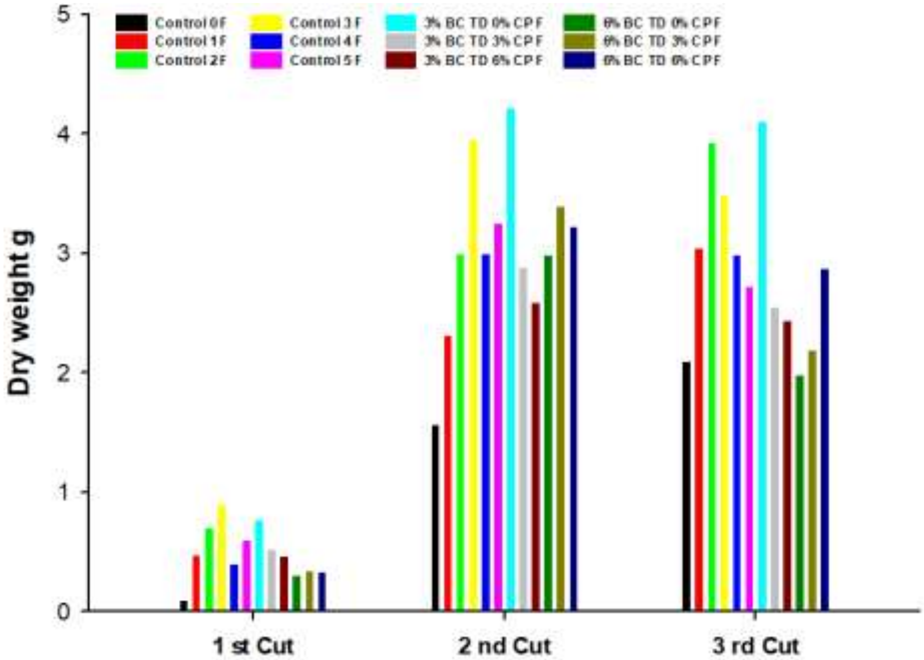


Fig. 12 Alfalfa dry weight in field for the first three cuts

"Six repetitions" BC = biochar (Feedstock source)

Our results showed that the nature and composition of the base impacts all the parameters of productivity. The addition of biochar to soils resulted in an increase in crop yield, soil microbial biomass and soil fertility, while soil pH also tended to increase, becoming less acidic as a result of the addition of biochar. However, There was no detectable relationship between the amount of biochar added and productivity (Biederman and Stanley Harpole 2013). Besides, the results suggested that biochar application increase forage yields and meet the forage safety standards by the dilution effect (Liu et al. 2020). Negative effects of biochar on productivity can be observed, usually due to the type of biomass, the cultivated plant and the type of soil (Jeffery et al. 2011; van Zwieten et al. 2010). Generally, the compost helps stabilize and increase crop productivity and quality, mainly due to the slow release of nutrients due to increased soil organic matter concentrations (Adugna 2016; Ejigu et al. 2022). Although the application of compost decreases the negative effects of salinity and heavy metals, the type of soil, the type and composition of compost and the application rate are the main parameters which judge the degree of this intervention (Mbarki et al. 2008; 2018). In all the growth parameters studied, the treatments with manure either without or with nitrogen fertilizers always give high productivity. Generally, the application of manure at reasonable rates does not have negative effects on alfalfa productivity or the environment. An application of three rates of manure 3,000; 6,000 and 12,000 gal/acre (comparable to our first two doses) before sowing significantly increases the productivity of alfalfa without negative effects (Schmitt et al. 1993). Generally, the application of organic fertilizers gives higher productivity of alfalfa than that of chemical fertilizers (Vasileva and Kostov 2015). The speed of maturity differs from treatment to treatment; this speed can indicate when the harvest gives better alfalfa quality. The degree of maturity of alfalfa is a prime parameter to judge forage quality, this parameter is directly related to the development stage of alfalfa. Early harvest causes low biomass of alfalfa, while late harvest causes high rate non-digestible fiber (Mueller 1989). The results of the study showed a non-equality and diversity of the results between the field and the pots, especially in the biochar treatments which showed significant difference in all the tests; this is mainly due to the weak germination in most of the doses which has affected growth and productivity. This is because the germination of seeds have crucial results in the development, productivity and all physiological parameters of the plant (Luo et al. 2018). Despite the ideas that can be drawn from pot tests on the effects of biochar on germination and plant growth, these results remain very limited and do not allow excessive generalization; a long-term field study will either endorse the pot results or reject them (Baronti et al. 2010).

Photosynthesis

Fig 13 and 14 shows measurements of photosynthesis under two cultivation conditions. The parameter is mainly linked to the physiology of the plant in a very precise moment which shows that a continuous monitoring is necessary in order to describe the behavior of alfalfa under different growth stages, substrate compositions and climatic conditions. Photosynthesis is generally linked to multiple parameters (environmental and climatic conditions, type of soil, light, plant, etc.) (El Moussaoui and Bouqbis 2022).

Photosynthesis is very sensitive to water stress. Khan et al. (2021) showed that water stress significantly decreased photosynthetic parameters. The application of biochar significantly attenuated the effects of water stress on the photosynthesis (Abideen et al. 2020). Biochar applied to soil under adequate water conditions significantly increased photosynthesis of maize plant (Tanure et al. 2019).

Conclusion

The closed system of the pots concentrates the toxic chemical elements and/or nutrients on the roots which intensify the effects of treatments on the various parameters of growth and productivity. Generally, the application of manure increases all parameters of alfalfa growth in pots and in the field. Although the high dose application of biochar and biocompost decreases productivity and limits all growth parameters, interesting positive results on productivity were noted with the 3% BC treatment in the field. Our study also shows that the results of pots do not give sufficient results for generalization. The results are approximate and depend on the conditions of culture, type of soil and type of substrate. What is strongly recommended is to test on the field the effect of biochar and biocompost at low dose during a dozen harvests to evaluate their long-term effects on the productivity of alfalfa, also the evaluation of the effect of these treatments on the growth behavior of cultivated alfalfa under stress.

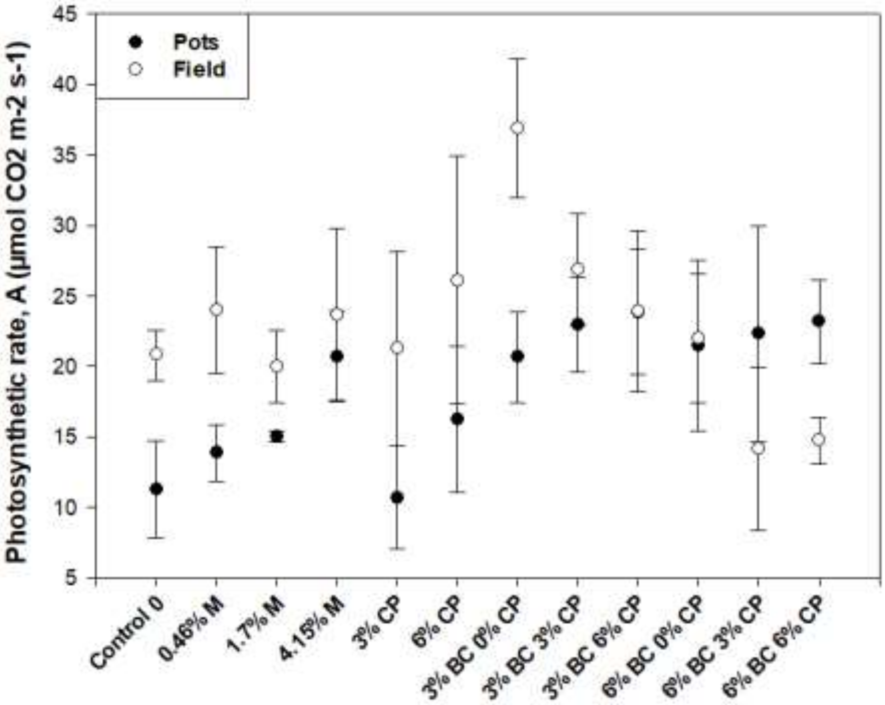


Fig. 13 IRGA Parameter; Photosynthetic rate, A (µmol CO2 m-2 s-1) for 1st measurement of Alfalfa leaf + standard deviation (n = 3)

BC = Biochar, CP = Bio-Compost, M = Manure

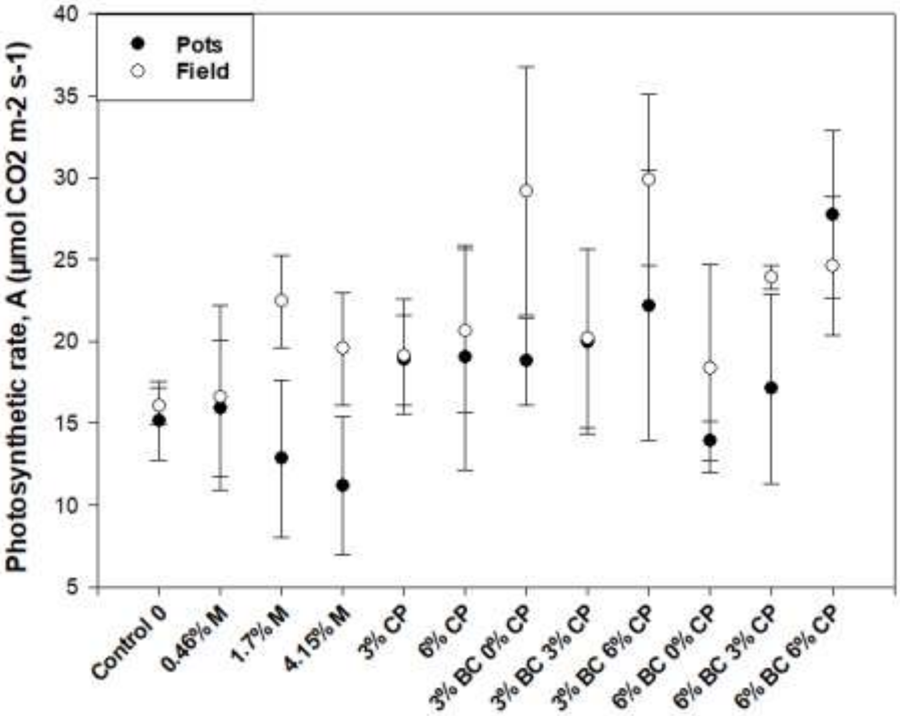


Fig. 14 IRGA Parameter; Photosynthetic rate, A (µmol CO2 m-2 s-1) for the 2nd measurement of Alfalfa leaf + standard deviation (n = 3)

BC = Biochar, CP = Bio-Compost, M = Manure

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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