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Interactions between humic substances and organic amendments affecting soil biological properties and growth of *Zea mays*.L in the arid land region

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Abstract

Application of organic amendments to the arid land soils is a sustainable tool to improve soil fertility. A greenhouse experiment was conducted to find the effect of humic acid (HA), alfalfa residues (AR) (*Medicago sativa* L.) and sheep manure (SM) on soil respiration, soil organic carbon content, soil structure, total bacteria, total fungi, and growth of corn plant *Zea mays*.L. Results showed that the highest aggregate stability was 44.2 % for the HA + SM treatment whereas it was 9 % for the HA treatment. The higher stability was associated with higher organic carbon 4.60%, and higher bacterial and fungal densities of 190×10^6 g^{-1} dry soil and 101×10^6 g^{-1} respectively. The HA + AR treatment did not increase aggregate stability significantly (27.7%), nor did the AR treatment (25.9%). Soil respiration over 70 days was significantly ($P < 0.05$) higher in the SM (42.9 CO_2-C mg g^{-1}) compared to the AR treatment (33.3 CO_2-C mg g^{-1}). In contrast, the HA + SM treatment had lower soil respiration

(27.4 CO_2-C mg g^{-1}) compared to the SM treatment (42.9 CO_2-C mg g^{-1}). The HA + AR treatment recorded higher plant weight (20.6 g) compared to HA (10.2 g) and control treatments (8.11 g). This study proved that humic acid did not enhance soil structure or the microbial community, whereas the combination of HA with SM or AR is more effective at promoting the soil microbial community, soil aggregation, organic carbon content and plant growth in the saline, arid land.

***Keywords*: humic acid, crop residues, sheep manure, soil respiration, total bacteria and fungi, organic carbon, soil structure, maize.**

Introduction

Soil organic matter is an important contributor to soil quality and productivity. Soil organic matter storage has a role in mitigating greenhouse gas emission effects on climate change (Garcia-Franco et al. 2018). Arid soils have low levels of organic carbon (Kneller et al. 2018) and offer limited physical protection to this organic matter due to lack of soil structure, vegetation and high temperature (AL-Maliki 2016). Sustainable agricultural practices are encouraged in the Arid, hot regions which in addition to low soil organic matter suffer from erosion, desertification, and salinity. The most common approach to enhancing soil fertility in these regions is to incorporate organic residues (e.g. green manures, farmyard manures, humic acid and crop residues) into the soil to increase the organic matter (OM) status (Metzger and Yaron 1987). There are considerable amounts of animal manures available for this purpose and these residues range in composition with 15-60% hemicellulose, 10-30% water cellulose, soluble fraction (5-30%), *alcohol-soluble fractions* (fats, oil, waxes, resins and pigments) and protein (Akhtar et al. 2014). The application of organic residues and sheep manure to soil increases microbial biomass, aggregate stability, soil microbial activity (Arocena et al. 2012; Al-Maliki 2012; AL-Maliki et al. 2017). Abiven et al. (2007) measured the microbial biomass carbon in the soil after adding cauliflower residues, wheat straw, cattle

manure and poultry woody compost and found marked increases in microbial biomass and aggregate stability compared to the control treatment. Likewise, Miao and Marrs (2000) found that the addition of farmyard manure to a soil in China led to increased organic matter content, total nitrogen and abundance of soil microorganisms (fungi and bacteria), as well as increased crop yield. Moreover, farmyard manure application can promote granular structure by increasing soil organic matter and biological activities (Arocena et al. 2012). Amendments of farmyard manure and poultry manure promoted the bacterial density and microbial activity in soils (Mishra et al. 2011).

There is a general agreement that the application of humic acids (HA) to the soil is beneficial to soil biological properties and growth of plants. Humic acids can increase the uptake of mineral elements, promoting root length and improving fresh and dry weights of crops (Mackowiak et al. 2001; Canellas et al. 2002; Chen et al. 2004). Furthermore, humic substances constitute a relatively stable fraction of carbon and improve water retention, pH buffering and thermal insulation (McDonnell et al. 2001). Similarly, humic acid may enhance soil structure. Piccolo et al. (1997) found that amending three soils with humic substances (equivalent to 100–200 kg ha^{-1}) improved aggregate stability and reduced substantially the disaggregating effects of water and drying cycles. Indeed, HA amendments markedly enhance plant growth under saline conditions. Türkmen et al. (2004) found the application of humic acid at 1000 mg kg^{-1} concentrations on saline soil increased seedling growth and nutrient contents of plants. Nevertheless, Türkmen et al. (2004) claimed that high levels of humic acid 2000 mg kg^{-1} limited plant growth and decreased nutrient contents. Likewise, Pukalchik et al. (2017) found the combination of humic substances with wood ash or wood biochar reduced microbial biomass carbon whereas the addition of humic substances (HS) alone increased microbial biomass in soil. Similarly, Gu and Doner (1993) recorded negative effects of humic acid on soil structure due to increased clay dispersability. Consequently, it

seems that the effect of humic acid on soil structure and microbial activity is not fully understood.

The proposed hypothesis of the current study is that the humic acid and the organic amendments enhance soil respiration, aggregate stability, organic carbon, soil bulk density, soil porosity, bacterial and fungal abundance, and the growth of corn. There have been no previous studies in the Arid - hot regions focusing on the combined use of humic acid, crop residues and sheep manure and their effect on these soil indices. So the present research was designed to study the combined effect of the addition of humic acid, crop residues and sheep manure on microbial activity, soil aggregation, and the corn plant yield.

Materials and Methods

Experimental site

A greenhouse experiment was carried out at the College of agriculture, AL-Qasim Green University, Iraq (32°23'34.3"N, 44°24'07.8"E). The average annual rainfall is 101 mm, the average annual temperature 30.1 ^{o}C with an average relative humidity is 49.4 %. The region is classed as an Arid –Desert-hot region (BWh) (Koppen and Geiger 1928). Some physical and chemical properties of the soil were determined: the texture was sandy loam (sand 60 %, clay 15 % and silt 25 %), pH 7.75, electrical conductivity 10.40 (dS/m) and the bulk density of the soil was 1.40 g/cm^3. Soil samples were taken from the top 30 cm depth of an adjacent field soil, then sieved to < 4 mm and 5 kg were placed in mesocosms.

Soil amendments and their composition

Alfalfa residues (*Medicago sativa* L.) were chopped to less than 1 cm and mixed thoroughly with 5 kg dry soil. Alfalfa residues and sheep manure were mixed into soils at a rate of 1 % (7 ton ha^{-1}) of the dry soil. The chemical compositions of sheep manure and alfalfa residues are shown in Table 1. Humic acid (1.1 g 142 kg ha^{-1}) was added to each 5 kg of soil in the irrigation water on three occasions during the growth of the plant. The first dose (0.3 g) was added before sowing, the second similar dose three weeks after planting. The third dose (0.4 g) was added five weeks after germination. DISPER Humic 85 % GS was used, a product containing humic and fulvic acids obtained from American Leonardite. The composition of the humic extract was 68 % humic acid, 17 % fulvic acid and 15 % potassium.

Six treatments (control (C), sheep manure (SM), alfalfa residues (AR), humic acid (HA), sheep manure + humic acid (SM+ HA) and alfalfa residues + humic acid (AR+ HA)) were replicated three times in a completely randomized block design with a total of 18 mesocosms. Mesocosms were incubated in the greenhouse for 30 days at 25 °C. Soil respiration was assessed for 30 days and the CO_2 release for 50 and 70 days. At the end of the period of incubation, the soil was analyzed to determine aggregate stability, microbial activity, the abundance of bacteria and fungi, bulk density, soil porosity and organic carbon.

Planting details

Seeds of maize (*Zea mays* L.) were sown at 3 cm depth on the 15th March 2015 with five hybrid seeds for each mesocosm. Seedlings were thinned to 3 seedlings at 14 days. Pots were weighed to maintain the soil moisture content close to 25 % field capacity. Fertilizers were uniformly applied to soil before planting. Urea was added at a rate of 300 kg/ha as a source of

nitrogen (equivalent to 0.75 g for each mesocosm). Compound fertilizer was applied at a rate of 120 kg/ha (triple superphosphate) as a source of phosphorous (0.3 g for each mesocosm), which contained 15 % calcium. Potassium sulphate was applied at a rate of 100 kg/ha (0.25 g for each mesocosm). The height and dry weight of maize were measured two months after planting.

Chemical analyses

Respiration was estimated based on the alkali trap method (Sonnenholzner and Boyed 2000). 20 g of soil was placed inside a flask corked with a stopper attached to a 10 ml glass bottle. The bottle was filled with a 5 ml solution of 0.62 M NaOH. The NaOH solution captured any CO_2 that was respired from the soil. For each molecule of CO_2 evolved during respiration, two molecules of NaOH are needed to neutralize the potential acidity.

1 M of HCl was used to titrate with the NaOH containing a phenolphthalein indicator. The endpoint of the titration was when the pink colour was changed. Soil respiration was estimated as follows:

$$\mathbf{CO2}\left(\frac{\mathbf{mg}}{\mathbf{g}}\right) = \frac{\mathbf{(B - V)N22}}{\mathbf{w}} \qquad \text{Equation 1}$$

Where B = standard HCl used to titrate NaOH in the blank (mL), V =standard HCl used to titrate NaOH in the treatment (mL), N = molarity of HCl (1.00 M), 22 = equivalent weight of CO_2, W = dry weight of soil in the chamber (g).

Aggregate stability was measured by wet sieving (Hazelton and Murphy 2007). The soil was prepared by sieving to 2 mm, and then transferred to sieves with 0.25 mm apertures on a shaking machine within a water tank. Resulting stable aggregates were dried at 110 °C and weighed, with correction for sand content. Soil microbial populations were processed using a soil dilution plate method (Nandhini and Josephine 2013). 1 g of soil was diluted with

sterilized water up to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}; 1 ml aliquots of each dilution were added to 20 ml of nutrient agar medium. Tryptic-soya agar was used to culture bacterial colonies and Martin's Rose Bengal agar for fungal colonies. Populations were enumerated by counting colony-forming units (CFU g^{-1} of dry soil). Bacterial plates were incubated at 28 °C for 3 days and fungal plates at 25°C for 7 days. To calculate the number of bacteria and fungi per gram of diluted sample, equation 2 was used:

Viable cells / g dry soil = (Mean plate count × Dilution factor) / Dry weight of soil (g) Equation **2**

Soil bulk density was determined using a core method (Culley 1993), 5 cm in length and 5 cm in diameter, with a core taken from the surface of each mesocosm. Cores were oven-dried at 105 C°, weighed to determine weight per given volume and the bulk density of each mesocosm calculated. Soil bulk density was calculated according to the below equation:

Soil bulk density= weight of soil (g)/ volume of soil (cm^3) Equation **3**

Porosity was calculated according to Głąb and Kulling, (2008) by dividing the soil bulk density to particle density to obtain averages values of the soil porosity according to the below equation:

$$\% \text{ porosity} = (1 - r_b/r_p) \times 100 \quad \text{Equation } \mathbf{4}$$

Where r_b= soil bulk density, r^p = particle density

A muffle furnace method was used to estimate soil organic carbon (Ball 1964; AL-Maliki et al. 2014). A Pyrex crucible containing 10 g of soil was oven-dried at 105 C° over-night, then placed into a muffle furnace and ignited at 400 °C for 16 hours. The percentage loss ignition

was calculated as follow: LOI results were divided by 1.724 to obtain the percentages of soil organic carbon.

$$\%\ \textbf{loss ignition} = \frac{(\textbf{oven dry soil weight} - \textbf{ignited soil weight})}{(\textbf{oven dry soil weight})} \times \mathbf{100} \qquad \text{Equation } \mathbf{5}$$

Statistical analysis

The data were analyzed using Minitab version 14. Microbial activity was analyzed using two-way analysis of variance (ANOVA), tested two factors (treatments and incubation periods). Results for the aggregate stability, bulk density, bacterial abundance, fungal abundance, organic carbon, dry weight of plant and height of plant were analyzed by one-way ANOVA. Means were further evaluated where significant treatment effects were observed using Tukey's honest significance difference (HSD) test with a significance level of $P < 0.05$. To test the relationships between variables, linear correlation coefficients were calculated at the significance level of $P < 0.05$.

Results

Effect of treatments on soil respiration (CO_2-C efflux) and microbial population

The amendment of alfalfa residues, sheep manure, and humic acid steadily and significantly increased soil respiration compared to the control treatment (Figure 1). The magnitude of increase was from 9.94 CO_2-C mg g^{-1} in the control treatment to 38.47, 42.92, 33.34, 27.44, and 26.11 CO_2-C mg g^{-1} for AR+ HA, SM, AR, SM+HA, and HA treatments respectively. Overall, the highest increase in soil respiration was in SM treatment, although it was not significantly different from the HA+ AR treatment. Overall, there was significantly lower soil respiration for the HA treatment compared to the AR +HA, SM, AR treatments. It was found

that soil respiration was markedly lower for the HA + SM treatment in a comparison to the SM treatment, whereas when HA treatment has combined with AR, soil respiration increased though not significantly compared to the AR treatment. Soil respiration was affected significantly by the incubation period; it was higher at 38.54 CO_2-C mg g^{-1} at day 50 compared to day 30 and 70 (23.45, and 26.27 CO_2-C mg g^{-1}) respectively. There was an interaction ($p<0.001$) (Figure 1) between time and treatments confirming that treatments did not affect soil respiration similarly with time. All treatments recorded a rise in soil respiration in day 50 and then a consistent decline to day 70, except for the HA treatment which showed a slight increase at day 50, then further increase at day 70. Moreover, the combination of HA with SM displayed a clear decline in soil respiration at day 50 and 70. In contrast, the (HA+ AR) treatment revealed a consistent increase in soil respiration over the incubation period.

On average, the bacterial abundance in the control treatment was significantly lower (15×10^6 g^{-1} dry soil) compared to (120×10^6 g^{-1} dry soil, 100×10^6 g^{-1} dry soil , 150×10^6 g^{-1} dry soil, 190×10^6 g^{-1} dry soil) the AR, AR+HA , SM and SM+HA treatments respectively (Figure 2). However, the bacterial population in the HA treatment was not significantly different from the control. A greater rise in the bacterial population was observed at (HA+SM) treatment (190×10^6 g^{-1} dry soil) and to less extent with the (HA+AR) treatment (100×10^6 g^{-1} dry soil). Total bacteria abundance was higher in the SM treatment compared to the AR treatment. The fungal abundance increased significantly in the AR, AR+HA, SM, and SM+HA treatments (Figure 3) compared to the control. In contrast, the fungal abundance in HA treatment was not significantly different from the control treatment. Furthermore, the fungal abundance increased in the AR compared to the SM treatment.

Effect of treatments on soil aggregation, soil bulk density and porosity

Aggregate stability varied markedly between treatments. A substantial significant increase was detected in the HA+SM treatment compared to other treatments (Figure 4). It was also noted that aggregate stability in the HA treatment was very low compared to other treatments and indeed lower than the control treatment; aggregate stability in the AR+ HA treatment was not significantly different from the control. The improvements in aggregate stability were clear comparing the HA+SM and AR treatments.

Soil bulk density was significantly lower in all treatments combinations compared to the control (Figure 5). Lower soil bulk density was detected in the HA+ AR treatment compared to all other treatments. The addition of the AR only did not significantly decrease soil bulk density compared to the SM, HA, AR+HA and SM+HA treatments.

Soil porosity varied among treatments (Figure 6). All treatments had a significant increase compared to the control. The highest increase in soil porosity was when HA was incorporated with the AR treatment. Soil porosity was lower in the AR treatment compared to the SM, HA, AR+HA, and SM+HA treatments.

Effect of treatments on soil organic carbon

Where soil was amended with organic matter, the soil organic carbon increased compared to the control (Figure 7). The highest increase was in the HA+SM treatment. Soil organic carbon content in the HA+ AR was not significantly different from the AR treatment. The HA only treatments led to significantly lower soil organic carbon than all other treatment combinations, although it was not significantly lower than the AR treatment. The soil organic carbon content in the SM treatment was significantly higher than in the AR treatment.

Effect of treatments on growth of the plant

Plant height was significantly greater in all treatments compared to the control (Figure 8). There were no significant differences in plant height between the SM, HA, AR+HA, and SM+HA treatments. The plant weight was significantly greater in all treatments compared to the control except for the HA treatment (Figure 9). There was a significant increase in plant weight when HA has combined with the AR and SM compared to the HA treatment and control.

The relationship between aggregate stability and biological binding agents

The correlations between aggregate stability and the biological parameters evaluated in this experiment may be related to improved soil structure and the plant growth. There was a highly significant correlation ($r = 0.82$, p-value < 0.001) between the aggregate stability and the bacterial abundance (Table 2). The correlation between aggregate stability and fungal abundance was also close ($r = 0.78$, p-value < 0.001). A significant correlation ($r = 0.74$, p-value < 0.001) was also found between soil organic carbon content and aggregate stability. Soil respiration was found to have a marginal non-significant correlation ($r = 0.40$, p-value = 0.06) with the aggregate stability.

Bulk density plays a vital role in enhancing soil quality and the plant growth. It was found that the soil respiration and the organic carbon content related to bulk density. A significant negative correlation ($r = -0.64$, p-value = 0.004) was found between soil respiration and bulk density. Furthermore, the correlation between organic matter and bulk density was significant ($r = -0.70$, p-value = 0.002). There were no significant correlations between bulk density and bacterial or fungal abundance.

The relationship between soil parameters and plant growth

Soil parameters may have affected plant growth. There was a significant negative correlation (r= - 0.78, p-value = 0.001) between the plant height and soil bulk density (Table 3). There were significant positive correlations (p-value < 0.001) between plant height and soil respiration, carbon and porosity. Fungal abundance had a weaker correlation with (r= 0.49, p-value = 0.03) plant height. In contrast, height was not correlated with bacterial abundance or aggregate stability.

Similar trends were found for plant biomass. with soil respiration, organic carbon content, porosity and aggregate stability positively correlated with biomass ($p < 0.01$) and bulk density negatively so ($p < 0.01$. There here was a significant correlation (r= 0.63, p-value = 0.005) between the fungal abundance and plant biomass, but not so for bacteria.

Discussion

Effect of treatments on soil respiration (CO_2-C efflux) and the microbial population

Soil respiration increased after addition of organic materials (Figure 1), probably due to the carbon, phosphorus and nitrogen inputs. Microbial decomposition of litter can yield fats, lignin, and waxes plus humus (Lynch and Bragg 1985; Christensen 1986; Angers et al. 1997). These findings are in accordance with those of Abiven et al. (2007) and AL-Maliki et al. (2017) who found a marked increase in soil microbial activity after the addition of organic residues. The highest increase in respiration for the SM treatment might have been due to its higher cellulose and lignin contents supporting microbial activity through slow early decomposition increasing thereafter. As expected (Dilly 2004), humic acid increased soil respiration because it is an energy source for the microbial activity. Also, added humic acid could lower the high pH of the saline soil favouring microbial activity. Microbes in the soil require a continuous supply of organic substrate. This increase in soil microbial activity at the

HA+AR could be a result of the larger quantities of nitrogen, phosphorous and carbon, and pH amelioration.

The incorporation of HA with SM decreased soil respiration compared to the SM treatment. This decrease in soil respiration could be linked to increases in aggregate stability and associated physical protection of organic matter (Sarker et al. 2018). There was an interaction effect for respiration (Figure 1) between treatments and time. A prominent feature of this was the increase at day 50 and subsequent decrease for most organic amendments contrasting with the HA treatment which showed a consistent rise to day 50 and 70; this was due probably to the chemical recalcitrance of the humic.

The bacterial and fungal populations increased in all the treatments compared to the control treatments (Figures 2 and 3). The highest increases in the bacterial and fungal abundance were for the HA + SM treatment. As with respiration, several factors including mineral nutrient contents, proportions of less labile organic fractions (e.g cellulose and lignin), pH effects and effects on soil structure may explain variations in treatment effects on bacterial and fungal abundance.

It was noted that the chemical composition of the organic materials played a crucial role in increasing the bacterial and fungal population in the soil. The bacterial population was higher in the SM treatment than the AR treatment. SM has large C/N ratio and contained more cellulose and lignin which might have decomposed slowly attracting and supporting the bacterial community over time. Soil bacteria can decompose cellulose in the aerobic and anaerobic soil circumstances (Coughlan and Mayer 1992). This study can witness that bacteria are important contributors to the soil cellulose degradation. Interestingly, the soil aggregate stability was higher in the SM treatment; this provides an extra protection for the bacterial community against the predator. Ranjard and Richaume (2001) found that bacteria

located within micropores in a good soil structure are protected against predation. Moreover, the stable soil aggregates have small pores impeding the diffusion of the water and oxygen giving a favour to the anaerobic bacteria to inevitably increase in the SM treatment. In these circumstances, the organic carbon in the small pores of the stable aggregates would not be accessible by the fungal population which has a larger size than bacteria, and they reside in the outer part of the soil aggregates, therefore they were relatively lower in the SM treatment than AR. In contrast, the fungal population was higher in the AR treatment which is from the plant origin containing more nitrogen, carbon and phosphorus which are used as an energy source to support the fungal activity. Fungi are important degraders in soils (Baldrian and Valášková 2008).

Effect of treatments on aggregate stability, bulk density and soil porosity

Figure 4 showed that the easily decomposable materials (AR with and without HA) did not significantly increase aggregate stability compared to the non-treated soil. In contrast, the highest increase in aggregate stability was in the more recalcitrant materials (SM with and without HA). The likely explanation is that the C/N ratio of litter residues affects soil aggregate stability dynamics. It is probable that low C/N ratio inputs of the AR treatment were decomposed between zero and 30 days, with short-term effects on aggregation; the higher C/N ratio SM inputs produced longer-term effects. Furthermore, humic acid would have mitigated the negative effects of high pH on microbial communities associated with improvements in soil structure. Interestingly, the higher bacterial and fungal abundance was in the SH+ HA treatment. Bacteria can produce polymers which might be adsorbed to soil surfaces leading to the formation of aggregates (Lynch and Bragg 1985). Here there was a close correlation ($r= 0.82$, p-value= 0.001) between aggregate stability and bacterial abundance. Moreover, fungal hyphae can link soil particles causing an enhanced soil

structure (AL-Maliki et al. 2017). Fungal hyphae might enmesh soil particles resulting in an improved soil stabilisation by acting as a ‘’sticky string bag’’ (Oades 1993; Haydu-Houdeshell 2018). These conclusions are consistent with the significant correlation (r= 0.78, p-value= 0.001) between aggregate stability and fungal abundance.

It was expected that humic acid would increase stable aggregates because it contains aliphatic and aromatic carbon are capable of linking soil particles together (Sung et al. 2012). However, this was not the case. Shanmunagathan and Oades (1983) and Oades (1984) observed that humic acid increased the negative charges on clays causing their dispersion. Disrupted soil structure could cause a reduction in the soil pores, potentially leading to anaerobic conditions and impeded activity of the soil microbes promoting soil structure. Here bacterial and fungal abundances were diminished when humic acid added to the soil. An increase in the microbial abundance can form binding agents resulting in a maximised aggregate stability. This result is consistent with Gu and Doner (1993) who found that humic acid was an effective dispersing agent for Na-clays and Na-soils. Likewise, Nelson et al. (1999) also revealed that the amino acids were responsible for clay dispersion in sodic soils.

The incorporation of organic materials in the soil led to higher soil porosity and lower bulk density (Figures 5 and 6). These findings are consistent with those of Rosolem et al. (2010) who found that soil organic matter enhanced aggregation and soil porosity, aeration, water infiltration, and water retention, and a decrease in soil bulk density. The largest improvement in porosity and bulk density was in the HA + AR treatment. This outcome could be attributed to the alfalfa residues being a high quality substrate for microbes producing binding agents such as fates, waxes and polysaccharides (Abiven et al. 2007). Reduced pH would have further enhanced this process. These results are in accordance with Franzluebbers (2002) who found that soil organic matter improved soil aggregation and macroporosity by enhancing the

soil biological activity. Similar results were recorded by Silveira Neto et al. (2006), who showed that soil bulk density decreased through an increase in soil organic matter.

Effect of treatments on soil organic carbon

Soil organic carbon content increased substantially after the incorporation of the organic materials (Figure 7). The largest increase was in the HA + SM treatment. Increases in organic carbon with inputs are inevitable but in this case may have been enhanced by higher aggregate stability in the SM + HA treatment protecting organic inputs from decomposition (Tisdall and Oades 1982; Ghosh et al. 2018). There was a close correlation (r =0. 70, p-value= 0.002) between aggregate stability and organic carbon. Furthermore, there was a higher consistent population of bacteria and fungi in the SM + HA treatment causing an improvement in soil organic matter decomposition leading to much more microbial mucilages leading to an obvious increase in soil organic carbon content. This point is proved by bringing a significant correlation (r =70, p-value= 0.001) (r =61, p-value= 0.006) between bacterial, fungal population and soil organic carbon respectively suggesting a particular role of the microbial population in increasing soil organic carbon. Fungi produce glomalin which is an important protein contributing to the organic carbon storage (Debode et al. 2016). Moreover, bacteria can produce mucilages and polysaccharides (Ahmed et al. 2018) which may also stabilise carbon inputs to some extent.

Effect of treatments on growth of the plant

All the organic materials significantly increased plant height and biomass (Figure 8). Organic materials contain large quantities of nutrients such as nitrogen, phosphorous and potassium which encouraged plant growth. Other indirect effects include improvements in soil structure, bulk density, soil porosity, microbial activity and organic carbon. The increase in aggregate stability and reduction in bulk density would have enhanced water supply to plants and promoted rooting thus increasing nutrient uptake and productivity (Yanardağ et al. 2017). These interpretations were consistent with correlations between plant and soil parameters (Table 3). Microbial activity plays a vital role in the conversion of organic nutrients into available plant forms. Organic carbon can promote the growth of the plant by improving the physical and biological health of soil as a source of energy (De Deyn et al. 2008). Improvements in physical conditions would also promote plant growth. Fungi have a potential to increase the soil fertility by mobilizing phosphorus, potassium and iron (Rashid et al. 2016). Fungal hyphae also enmesh soil particles, leading to an enhanced soil structure (Debode et al. 2016; AL-Maliki et al. 2017; Ghorchiani et al. 2018).

Conclusion

This study concluded that the application of HA alone did not improve aggregate stability and bacteria and fungi abundance in a saline soil. The incorporation of SM with HA improved all of the above parameters and in addition increased organic carbon. The combination of HA with AR improved soil respiration, bulk density, soil porosity and plant dry weight. Soil respiration was lower in HA + SM treatment with evidence of organic carbon protection. The increases in plant growth with organic inputs were attributed, in addition to their nutrient inputs, to improved physical conditions and microbial activity. This study suggests that the application of HA alone could not improve arid land soil. However, the combination of HA with SM or AR might be a potential means of achieving this objective with benefits for the growth of plants in arid ecosystems which suffer from salinity.

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References

Abiven, S., Menasseri, S., Angers, D., & Leterme, P. (2007). Dynamics of aggregate stability and biological binding agents during decomposition of organic materials. *European Journal of Soil Science, 58*(1), 239-247.

Ahmed, M.A., Banfield, C.C., Sanaullah, M., Gunina, A., Dippold, M.A., 2018. Utilisation of mucilage C by microbial communities under drought. *Biology and Fertility of Soils* 54, 83-94

Akhtar, K., Shah, S. N. M., Ali, A., Zaheer, S., Wahid, F., Khan, A., et al. (2014). Effects of humic acid and crop residues on soil and wheat nitrogen contents. *American Journal of Plant Sciences, 5*(09), 1277.

AL-Maliki, S. (2012). *The effect of earthworms, litter quality and atmospheric CO2 on soil aggregation and associated microbial indices. PhD thesis,* Aberystwyth University , Wales.

AL-Maliki, S. (2015). Abundance of bacterial and fungal community after amendments of organic residues in some soils from Babylon province. *Karbala Journal of Agriculture, 2*, 63-76

AL-Maliki, S. (2016) . Response of microbial community in the arid land to wheat residues and soil compaction. *Journal of Kerbala University*, *14*, 114-123

Al-Maliki, S., & Scullion, J. (2013). Interactions between earthworms and residues of differing quality affecting aggregate stability and microbial dynamics. *Applied soil ecology, 64*, 56-62.

AL-Maliki, S. M., Jasim, H. A., AL-Watefi, A. S., & Abdulabbas, A. A. (2015). Effect of tillage and no-tillage on aggregate stability, organic matter content and microbial activity in the Babylon province, Iraq. *Envis Newsletter, 4*, 2-6

Al-Maliki, S. M., Jones, D. L., Godbold, D. L., Gwynn-Jones, D., & Scullion, J. (2017). Elevated CO2 and Tree Species Affect Microbial Activity and Associated Aggregate Stability in Soil Amended with Litter. *Forests, 8*(3), 70.

Angers, D., Recous, S., & Aita, C. (1997). Fate of carbon and nitrogen in water-stable aggregates during decomposition of 13C15N-labelled wheat straw in situ. *European Journal of Soil Science, 48*(2), 295-300.

Arocena, J., Mourik, J. v., & Cano, A. F. (2012). Granular soil structure indicates reclamation of degraded to productive soils: a case study in southeast Spain. *Canadian Journal of Soil Science, 92*(1), 243-251.

Baldrian, P., & Valášková, V. (2008). Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiology Reviews, 32*(3), 501-521.

Ball, D. (1964). Loss-on-ignition as an estimate of organic matter and organic carbon in non-calcareous soils. *European Journal of Soil Science, 15*(1), 84-92.

Berg, B., Hannus, K., Popoff, T., & Theander, O. (1982). Changes in organic chemical components of needle litter during decomposition. Long-term decomposition in a Scots pine forest. I. *Canadian Journal of Botany, 60*(8), 1310-1319.

Christensen, B. T .(1986) .Straw incorporation and soil organic matter in macro-aggregates and particle size separates. *European Journal of Soil Science, 37*(1), 125-135.

Coughlan, M., & Mayer, F. (1992). The cellulose-decomposing bacteria and their enzyme systems. In A. Balows, H. Trueper, M. Dworkin, W. HarderW & K. Schleifer (Eds.), *The prokaryotes: a handbook on the biology of bacteria*. New York: Springer-Verlag.

Culley, J. L. B. (1993). Density and compressibility. In M. Carter (Ed.), *Soil Sampling and Methods of Analysis.* (pp. 529–539). Florida, USA: Lewis Publishers, Boca Raton, Florida.

Debode, J., De Tender, C., Soltaninejad, S., Van Malderghem, C., Haegeman, A., Van der Linden, I., Cottyn, B., Heyndrickx, M., Maes, M., 2016. Chitin mixed in potting soil alters lettuce growth, the survival of zoonotic bacteria on the leaves and associated rhizosphere microbiology. *Frontiers in microbiology 7*, 565.

De Deyn, G.B., Cornelissen, J.H., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology letters* 11, 516-531.

Dilly, O., 2004. Effects of glucose, cellulose, and humic acids on soil microbial eco-physiology. *Journal of plant nutrition and soil science 167*, 261-266.

Franzluebbers, A. (2002). Water infiltration and soil structure related to organic matter and its stratification with depth. *Soil and Tillage Research, 66*(2), 197-205.

Garcia-Franco, N., Hobley, E., Hübner, R., Wiesmeier, M., 2018. Climate-Smart Soil Management in Semiarid Regions, *Soil Management and Climate Change*. Elsevier, pp. 349-368.

Głąb, T., Kulig, B., 2008. Effect of mulch and tillage system on soil porosity under wheat (*Triticum aestivum*). *Soil and Tillage Research 99*, 169-178.

Ghosh, A., Bhattacharyya, R., Meena, M., Dwivedi, B., Singh, G., Agnihotri, R., Sharma, C., 2018. Long-term fertilization effects on soil organic carbon sequestration in an Inceptisol. *Soil and Tillage Research 177*, 134-144.

Ghorchiani, M., Etesami, H., Alikhani, H.A., 2018. Improvement of growth and yield of maize under water stress by co-inoculating an arbuscular mycorrhizal fungus and a

plant growth promoting rhizobacterium together with phosphate fertilizers. *Agriculture, Ecosystems & Environment* 258, 59-70

Gu, B., & Doner, H. E. (1993). Dispersion and aggregation of soils as influenced by organic and inorganic polymers and inorganic polymers. *Soil Science Society of America Journal, 57*(3), 709-716.

Hazelton, P., & Murphy, B. (2016). Interpreting soil test results: what do all the numbers mean?: CSIRO publishing.

Haydu-Houdeshell, C.-A., Graham, R.C., Hendrix, P.F., Peterson, A.C., 2018. Soil aggregate stability under chaparral species in southern California. *Geoderma 310,* 201-208.

Kneller, T., Harris, R.J., Bateman, A., Muñoz-Rojas, M., 2018. Native-plant amendments and topsoil addition enhance soil function in post-mining arid grasslands. *Science of the Total Environment 621*, 744-752.

Köppen, W., & Geiger, R. (Cartographer). (1928). *Climate Classification world map*

Lynch, J., & Bragg ,E. (1985). Microorganisms and soil aggregate stability. In *Advances in soil science* (pp. 133-171): Springer.

Magill, A. H., & Aber, J. D. (1998). Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. *Plant and Soil, 203*(2), 301-311.

Malik, K. A., & Azam, F. (1985). Effect of humic acid on wheat (Triticum aestivum L.) seedling growth. *Environmental and Experimental Botany, 25*(3), 245-252.

McDonnell, R., Holden, N., Ward, S., Collins, J., Farrell, E., & Hayes, M. (2001). *Characteristics of humic substances in heathland and forested peat soils of the Wicklow Mountains.* Paper presented at the Biology and Environment: Proceedings of the Royal Irish Academy.

Metzger, L., & Yaron, B. (1987). Influence of sludge organic matter on soil physical properties. In *Advances in soil science* (pp. 141-163): Springer.

Miao, Z., & Marrs, R. (2000). Ecological restoration and land reclamation in open-cast mines in Shanxi Province, China. *Journal of environmental management, 59*(3), 205-215.

Mishra, C., Chhotaray, D., Rath, M., & Behera, J. (2011). Studies on the effect of organic amendments and earthworm inoculation on some important quality parameters of iron mine spoil. *STUDIES, 6*(1), 29-32.

Nachtergaele, J., & Poesen, J. (2002). Spatial and temporal variations in resistance of loess-derived soils to ephemeral gully erosion. *European Journal of Soil Science, 53*(3), 449-463.

Nandhini, B., & Josephine, R. M. (2013). Original Research Article A study on bacterial and fungal diversity in potted soil. *International Journal of Current Microbiology and Applied Science, 2*(2) 1-5.

Nelson, P., Baldock, A., Oades, J., Churchman, G., & Clarke, P. (1999). Dispersed clay and organic matter in soil: their nature and associations. *Soil Research, 37*(2), 289-316.

Oades, J. M. (1984). Soil organic matter and structural stability: mechanisms and implications for management. In *Biological Processes and Soil Fertility* (pp. 319-337): Springer.

Piccolo, A., Pietramellara, G., & Mbagwu, J. (1997). Use of humic substances as soil conditioners to increase aggregate stability. *Geoderma, 75*(3-4), 277-267

Pukalchik, M., Mercl, F., Panova, M., Břendová, K., Terekhova, V.A., Tlustoš, P., 2017. The improvement of multi-contaminated sandy loam soil chemical and biological properties by the biochar, wood ash, and humic substances amendments. *Environmental pollution 229*, 516-524.

Rashid, M. I., Mujawar, L. H., Shahzad, T., Almeelbi, T., Ismail, I. M., & Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiological research, 183*, 26-41.

Ranjard, L., Richaume, A., 2001. Quantitative and qualitative microscale distribution of bacteria in soil. *Research in microbiology* 152, 707-716.

Rosolem, C. A., Sgariboldi, T., Garcia, R. A., & Calonego, J. C. (2010). Potassium leaching as affected by soil texture and residual fertilization in tropical soils. *Communications in soil science and plant analysis*, *41*(16), 1934-1943.

Sarker, J.R., Singh, B.P., Cowie, A.L., Fang, Y., Collins, D., Badgery, W. and Dalal, R.C., 2018. Agricultural management practices impacted carbon and nutrient concentrations in soil aggregates, with minimal influence on aggregate stability and total carbon and nutrient stocks in contrasting soils. *Soil and Tillage Research*, *178*, 209-223.

Silveira-Neto, R & ,.Azzoni, C. R. (2006). Location and regional income disparity dynamics: The Brazilian case. *Papers in Regional Science*, *85*(4), 599-613.

Shanmuganathan, R., Oades, J., 1983. Influence of anions on dispersion and physical properties of the A horizon of a red-brown earth. Geoderma 29, 257-277.

Sonnenholzner, S., & Boyd, C. E. (2000). Vertical gradients of organic matter concentration and respiration rate in pond bottom soils. *Journal of the World Aquaculture Society*, *31*(3), 376-380.

Sung, C.T.B., 2012. Aggregate stability of tropical soils in relation to their organic matter constituents and other soil properties. *Pertanika Journal of Tropical Agricultural Science 35*, 135-148.

Tan, K. H. (1994). *Environmental soil science*. New York, USA: Marcel Dekker Inc.

Tisdall, J., Cockroft, B., & Uren, N. (1978). The stability of soil aggregates as affected by organic materials, microbial activity and physical disruption. *Soil Research, 16*(1), 9-17.

Tisdall, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. *European Journal of Soil Science, 33*(2), 141-163.

Türkmen, Ö., Dursun, A., Turan, M., & Erdinç, Ç. (2004). Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (*Lycopersicon esculentum L.*) seedlings under saline soil conditions. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 54*(3), 174-168 .

Yanardağ, I., Zornoza, R., Bastida, F., Büyükkiliç-Yanardağ, A., García, C., Faz, A., Mermut, A., 2017. Native soil organic matter conditions the response of microbial communities to organic inputs with different stability. *Geoderma 295*, 1-9.