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Amelioration of Organic Carbon and Physical Health of Structurally Disturbed Soil through Microbe–Manure Amalgam

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Abstract: Less precipitation, high temperature, and minimal natural vegetation are characteristic of regions having an arid climate. The harsh environment massively destructs the soil structure of that area by burning soil organic carbon, leading to deteriorated soil nutritional quality, creating a significant threat to agricultural production and food security. Direct application of organic wastes not only substitutes lost organic carbon but also restores soil structure and fertility. This study was conducted to assess the impact of organic amendments, i.e., farm manure (FM), poultry manure (PM), molasses (MO), and Exo-Poly Saccharides (EPS) producing rhizobacterial strains i.e., M_2, M_{19}, M_{22} amalgams as treatments. To assess the impact of treatments on soil carbon and structure restoration to hold more water and nutrients, a 42-day incubation experiment using a completely randomized design (CRD) under the two-factor factorial arrangement was conducted. Macro aggregation (0.25 to >1 mm), carbon retention in macro aggregates, active carbon (dissolved organic carbon, a mineral-associated organic carbon, microbial biomass carbon), total organic carbon, the carbon mineralization activities, and water retention capacities were observed to be highest in soils that were treated with (FM + M_2, FM + M_{22}, PM + M_{19}, and MO + M_{19}). Finally, we conclude that organics mineralization by microbial actions releases organic glues that not only impart particle aggregation but also conserve organics as aggregate entrapped carbon. Amalgamated application of microbe–manure combinations directly impacts soil structure and organic carbon contents, but in an indirect scenario, it improves the fertility and productivity of the soil. Therefore, it is strongly recommended to use organic manures and microbes in combination to restore structurally degraded lands.

Keywords: rhizobacteria; organic manures; soil; macro aggregate; carbon; water

1. Introduction

Soil physio–biochemical characteristics and functioning of terrestrial ecosystems are pivoted around soil carbon [1,2]. Soil carbon retention and turnover balance are crucial to sustainable agricultural systems, productivity, fertility, and the structure of the soil. Appropriate management practices assert soil strategic sink for atmospheric CO_2 to regulate the

global carbon (C) cycle [3–7]. An increase in agricultural throughput during recent decades owes to increased fertilization and pesticide that destroyed the environment [8]. Plant productivity and ecosystem utilities (soil structure, nutritional capacity, sequestered C, nutrient cycling, and hydrological amenities) were ruined during the last century because of a 30–50% decline in soil carbon [9]. Minimal till, no fallow, crop rotations, and judicious input use aim to mitigate negative impacts to sustain production [8–10]. Higher plants govern primary production in terrestrial ecosystems utilizing the atmosphere's CO_2, nevertheless, soil microbiota regulates carbon budgets via copious roles in soil carbon buildups thus amending nutrient availability and driving longevity and solidity of carbon pools [5,7]. Maneuverings of the agro-ecosystems are chief drivers of carbon cycling by changing microbial community structure [3,5,8,11]. Understanding of systematic management of soil carbon is a key task for predicting carbon dynamics under various management practices.

Manuring had been a common practice in China, Japan, and Korea for nearly 4000 years to increase soil organic matter (SOM) to restore soil fertility for attaining adequate yield. Nutrient stream, soil physical vigor, erosion protection, and biological activity are contributed by SOM [12]. Mineral fertilization secondarily increases soil carbon sequestration [13], since organics, either alone or in combination with mineral fertilizer, are more effective in improving SOM and its segments than mineral nourishment alone [14]. Artificial fertilization helps in aggregate materialization [15] and stabilization [16] augmenting spatial inaccessibility for decaying microbes [17].

Organics enhance soil nutritional capacity mainly attributable to stabilized soil structure [18] through soil biochemical alterations [19,20]. For instance, soil organic carbon [21], carbon sequestration [22], microbial biomass and activities [23], and release of organic glues [20] to formulate and stabilize aggregate [24] are caused by organic application to soil. Studies by Zhang et al. [25], Liang et al. [26], and Huang et al. [27] testified a strong correlation of soil structural stability with soil organic carbon that releases particle binders on microbial decomposition being considered the most important driver during the formation and stabilization of aggregates.

The aggregated structure produced by decaying organisms, saccharide excretions of living entities, and cohesive bonds of soil particles with organics are responsible for carbon storage in terrestrial regions [28] as it regulates microbial decomposition rates [29]. Microbial biomass is a more active particle binding fraction than SOM [3,20,22]. Nevertheless, biomass distribution within aggregates is still inconsistent [30,31] which may be accredited to pore size distribution or aggregate carbon content [22]. Soil structure and chemical properties mediate carbon storage [32,33] by entrapping it in aggregates, making it inaccessible to degrading microbes and extracellular enzymes. Aggregation creates ecological niches varying in physiochemical and structural characteristics promoting colonization and grouping of microbial communities in each aggregate [34]. Familiarity with the activities of microbiota in aggregates is presently poor but necessary to consider the regulation of soil carbon to increase production and sustain agriculture [35]. Although aggregate stability is strongly correlated with SOM and microbial biomass, it is still uncertain whether their relationship is aggregate scale-dependent or relies on aggregate size.

Knowledge of the relationship between SOM and microbial biomass as soil binders within aggregates would be helpful to improve soil structure and fertility. Therefore, this study was envisioned to evaluate the impact of organic amendments and EPS-producing bacterial strains on soil aggregation, aggregate-associated soil organics, and microbial biomass carbon to clarify the relationship among soil organic carbon, microbial biomass carbon, and aggregate stability.

2. Materials and Methods

The soil was collected from the research area of the Arid Zone Research Center (Latitude 31°88′0″ N and longitude 70°86′0″ E), Pakistan Agricultural Research Council, Dera Ismail Khan, KP, and Pakistan. The area has a typical tropical monsoon climate with <250 mm mean annual precipitation and 32 °C mean annual temperature, respectively.

Above 10 °C cumulated mean annual temperature is 35.30 °C while 80% precipitation is estimated from March to September.

Soil samples were collected from a 0–15 cm depth using grid sampling with 3 m squares during the autumn of 2018, mixed to get a homogenized composite sample. Plant roots and other debris were removed before sieving and grinding. To quantify macro-aggregate development, incubation of soil was initiated with micro-aggregates (<0.25 mm) and smaller texture fractions (silt and clay) by separating them using a <0.25 mm mesh-sized sieve and the larger-sized aggregates were ground using grinder @ 1 sample per minute, shaken through <0.25 mm sieve. Soil samples contained 5.7 g kg^{-1} SOC, 0.85 g kg^{-1} N, 6.7 (C: N), 7.9 units of pH, clay, sand, and silt (%) having clay loam texture, and were calcareous. Microbial strains (M_2, $M_{19,}$ and M_{22}) having high exopolymer production potential [36] (Table 1) and organic amendments (Farm Manure, Poultry Manure, and Molasses) were used as treatment combinations.

Table 1. Exopolysaccharide secreting potential of microbes used for the study.

Microbes	EPS Yield g L^{-1}	EPS Chemical Composition (g L^{-1})				Monosaccharides (g L^{-1})						
		Carbohydrate	Protein	Acetyl Residues	Sulfates	Glucose	Mannose	Rhamnose	Galactose	Arabinose	Xylose	Fucose
M_2	5.274	3.458	0.09	0.183	11.3	1.468	1.518	0.130	0.184	ND	0.03	ND
M_{19}	5.534	3.571	0.022	2.051	3.12	1.108	1.81	0.164	ND	0.03	ND	ND
M_{22}	6.831	4.512	0.199	1.101	8.597	1.87	2.01	0.206	0.03	ND	0.20	ND

ND (Not detected) "Reprinted/adapted with permission from Ref. [36]. 2021, Haroon Shehzad".

3. Manure Composition

Organic manures were ground and sieved to <2 mm. They were analyzed for nutritional (NPK) contents, organic carbon contents, and water retention % age by using standard analytical procedures. Soil total nitrogen was calculated by Bremner [37] Kjeldahl's method. Di-acid (HNO_3 and $HClO_4$) mixture was used to digest samples. Digested samples were run on the spectrophotometer to calculate phosphorus contents [38]. Potassium was calculated by running the digested samples on the flame photometer [38]. Physiochemical ratios of these organic amendments are presented in Table 2.

Table 2. Physicochemical characteristics of organic amendments used for the study.

Property	pH	EC	WHC	C	N	P	K
Unit		dS m^{-1}	%	%	%	%	%
FM	6.9 ± 0.07	2.6 ± 0.03	43.1 ± 2.1	34.02 ± 2.45	0.67 ± 0.07	2 ± 0.14	0.12 ± 0.01
PM	6.3 ± 0.10	3.5 ± 0.01	45.3 ± 1.23	25.67 ± 2.12	1.12 ± 0.13	1.03 ± 0.09	0.19 ± 0.01
MO	6.1 ± 0.09	1.1 ± 0.02	39.87 ± 1.24	28.1 ± 2.06	0.51 ± 0.07	2.67 ± 0.18	0.49 ± 0.01

WHC (Water Holding Capacity), C (Carbon), N (Nitrogen), P (Phosphorus), K (Potassium), FM (Farm Manure), PM (Poultry manure), and MO (Molasses).

3.1. Incubation Experiment

Air dried soil samples (250 g) were sterilized at 121 °C, mixed with blends of organic amendments and microbial strains were placed in cylindrical, flat-based plastic containers with 3.8 cm inner diameter, 5.5 cm outer diameter, 15.5 cm height, and 70 µm × 140 µm orifices in walls. Each cup was aerated with pipes connected to aquarium aeration pumps. Nutrition and irrigation were supplied. A solution of 1 N KOH in a cock sealed conical flask was attached to capture microbe respired CO_2 [39]. 100% water retention capacity of the soil was maintained according to the method [40]. Unamended soil was considered to control and was processed as with treated soil. Cups were sealed with lids and incubated in the Hettich incubator (HettCube 200R) on 18 November 2018. Three replicates of each treatment were non-destructively sampled on 2 December 2018, 16 December 2018, and 30

December 2018, for analysis. Sampled soil was air dried, weighed, and then used for soil aggregate fractionation.

3.2. Soil Aggregate Extraction

Wet soil (<2 mm) was sieved through 106, 250, 500, and 1000 µm mesh-sized sieves to fractionate soil aggregates using the method of Six et al. [41]. The Soil Aggregate Analyzer (Model SAA 8052) was used for fractionation (Model SAA 8052). Soil samples were passed through a 2 mm sized sieve and were soaked in DI water and kept overnight at normal temperature (20 ± 2 °C). A series of sieves were racked upon each other with the largest mesh sized on top and were suspended in the water container. The time of complete up and down cycles was adjusted to 30 times per minute and the sieves were placed so that one inch of the top sieve was out of water when going down. Then these pre-soaked soil samples were poured on top of the 1000 µm sized sieve. The sieving cycle was then started with only 3 cm up and down the distance. Samples were then collected from all the sieves and containers, oven-dried at 60 °C (avoid burning of organic matter), and weighed. The percent aggregates were then calculated from weighted samples. Oven-dried samples were subjected to subsequent organic carbon fractionation.

3.3. Organic Carbon Fractionation

Organic carbon total, carbon different sized water stable aggregate, and other organic carbon fractions were assessed through wet oxidation with $K_2Cr_2O_7$ at 120 °C for an hour in presence of sulphuric acid, and the solution's color intensity was measured at 578 nm wavelength using UV visible spectrophotometer (Hitachi U-2000) [42].

Organic carbon fractions were separated as per the method described by Six et al. [42]. A 5 g (<2 mm) soil sample was immersed in 35 mL of 1.85 g mL^{-1} NaI solution in a centrifuge tube of 50 mL volume and tubes were gently shaken by hand several times and remained materials on the inside of the wall were washed with 10 mL of NaI to make 50 mL volume. Air was exhausted by placing in a vacuum for 10 min, equilibrated for 15 min, and centrifuged for one hour @ 2000 rpm. The supernatant was passed through a 0.45 µm membrane and dissolved organic carbon content was measured. Samples passed through the filter were dispersed in 5 g L^{-1} Na-hexametaphosphate for 18 h of continuous shaking on the reciprocal shaker. The dispersed segment was passed through 53 µm sieves to collect mineral-associated organic carbon (mSOC), <53 µm, dried at 60 °C, weighed, and analyzed for organic carbon contents.

3.4. Soil Biological Activity

Microbial activity was assessed by calculating the total evolved CO_2 during the experiment. Regarding the study, the modified Zibilski [39] method was used to calculate the total CO_2 produced. Each container was connected with a flask containing 1 N KOH solution through a connecting tube. A total of 1 mL 50% $BaCl_2$ was added to the flask to precipitate carbonates as insoluble barium carbonates. 2–3 drops of phenolphthalein were added as indicators and the solution was titrated against 1 N HCl solution until a colorless endpoint. *Evolved* CO_2 was calculated by using the formula

$$Evolved\ CO2 = (B - V)NE \quad (1)$$

V is the volume of acid required for titration of alkali attached with amended soil, B is the required acid volume for titration of alkali attached to unamended control, N is the normality of acid (1 N), and E is the equivalent weight of CO_2 (22).

3.5. Soil Water Retention Capacity

Soil water retention capacity was measured by pre-defined matric potential [43] with the help of suction plates at 0.3, 0.6, 1.0, 3.0, and 4.5 bar pressure, and a linear regression equation was calculated by using ln (h) versus $ln\ \theta/\theta_s$ to find water contents at field

capacity (θ_{FC}) and permanent wilting point (θ_{PWP}) of soil [44]. The following equation was developed by using ln (h) versus ln θ/θ_s to get (θ_{FC}) and (θ_{PWP}) etc.

$$ln\ P = ln\ P_{\alpha} + b\ ln\ (\theta/\theta_s) \quad (2)$$

P is matric potential (k Pa), "P_e" (intercept) is air entry value/bubbling pressure that has an inverse relation with "α", and "*b*" is the slope of $ln\ P$ vs. θ/θ_s of the water retention curve. The linear relationship between $ln\ \theta/\theta_s$ [–] and ln (*P*) [kPa] was observed for experimental soil with an intercept (0.0211) and a negative slope of −7.2615 (Figure 1). Water retention properties of the experimental soil are presented in Table 3.

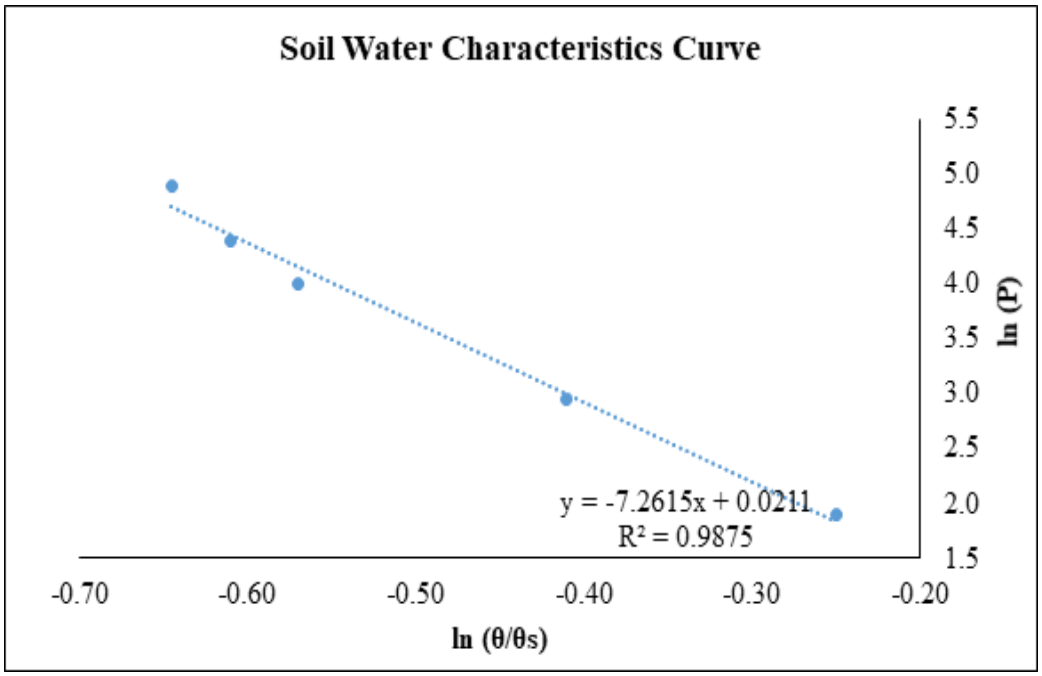

Figure 1. Soil water characteristics curve.

Table 3. Water retention properties of soil used for pot study.

Water Retention Properties	Θ_S	Θ_{FC}	Θ_{PWP}	Θ_{AWC}
Units		**(%)**		
	45.8 ± 0.93	23.68 ± 0.63	11.21 ± 1.02	12.47 ± 0.79

Data are an average of three replicates with standard error.

3.6. Statistical Analysis

Statistically, all data were presented as means of three replicates with standard error. Preferentially best-performing treatments will be exemplified using multivariate cluster analysis (Minitab-17®).

4. Results

The experimental soils were treated with different EPS secreting rhizobacterial strains and organic substrates while the moisture and temperature of these treated soils were maintained at 100% of soil WHC and 32 °C, respectively. Table 4 explicates that the bioaugmentation of soil with EPS secreting rhizobacterial strains in the presence of artificially applied organic substrates resulted in stabilized soil structure than non-treated soils. The proportion of aggregate size distribution varied with the duration of the experiment and treatments (Table 4). Regarding treatments, small-sized macro aggregates (0.25–0.5 mm)

and macro aggregates (0.5–1 mm) dominated in treated soils, respectively. In T_1 large macro aggregates were highest at 15.69, 15.99, and 16.68% which was at par with T_3 and T_6 but is suggestively higher than other treatments, especially the control. Macro aggregates (0.5–1 mm) proportion of 18.52, 19.98, and 20.53% was dominated in T_3 was two folds more than the control but was at par with all treatments except T_4 and T_5. Small macro aggregates (0.25–0.5 mm) were dominated (29.53, 30.62, and 31.71%) in T_3, which was statistically similar to T_1, T_4, T_5, and T_6. The proportion of small macro aggregates was 59.8, 64.27, and 67.25% more in T_4 than in untreated soil. Meso aggregates (0.106–0.25 mm) percentage was least observed in all treated soils that go on declining with incubation duration, the smallest amount was found in T_4 at the start, but its decreasing trend was slower than in T_3 and other treatments. Dispersed particles (<0.106 mm) were highest in untreated soils and increased non-significantly over time while particle dispersion was least in T_1 also having a declining trend with the duration of the experiment and the same procedure of decrement was observed in all treated soils.

Table 4. Organic substrates and microbial amalgams affect water stable aggregation and aggregate carbon retention.

Aggregate Size (mm)	Treatments	Organic + Microbe	Water Stable Aggregates (%)			Aggregate Organic Carbon (g kg^{-1})		
			Days after Incubation			Days after Incubation		
			14th	28th	42nd	14th	28th	42nd
>1 mm	T_0	CTRL	4.39 ± 0.15	4.34 ± 0.12	4.39 ± 0.12	1.62 ± 0.01	1.69 ± 0.02	1.75 ± 0.00
	T_1	FM + M2	15.69 ± 0.61	15.99 ± 0.68	15.69 ± 0.69	3.23 ± 0.06	3.31 ± 0.17	3.36 ± 0.11
	T_2	FM + M19	12.92 ± 0.57	13.21 ± 0.97	12.92 ± 1.04	3.35 ± 0.04	3.43 ± 0.16	3.48 ± 0.11
	T_3	FM + M22	14.25 ± 0.66	14.50 ± 0.37	14.25 ± 0.35	3.23 ± 0.05	3.27 ± 0.12	3.32 ± 0.12
	T_4	PM + M2	13.48 ± 0.34	13.76 ± 0.67	13.48 ± 0.72	3.29 ± 0.07	3.36 ± 0.06	3.42 ± 0.05
	T_5	PM + M19	12.66 ± 0.32	12.90 ± 0.29	12.66 ± 0.30	3.26 ± 0.09	3.33 ± 0.13	3.39 ± 0.15
	T_6	PM + M22	13.65 ± 0.64	13.93 ± 0.85	13.65 ± 0.93	3.23 ± 0.06	3.31 ± 0.19	3.36 ± 0.14
	T_7	MO + M2	14.02 ± 0.33	14.31 ± 0.64	14.02 ± 0.72	2.91 ± 0.09	2.98 ± 0.08	3.02 ± 0.02
	T_8	MO + M19	13.84 ± 0.16	14.12 ± 0.50	13.84 ± 0.57	3.01 ± 0.06	3.08 ± 0.09	3.12 ± 0.02
	T_9	MO + M22	13.63 ± 0.40	13.920.77	13.63 ± 0.85	2.88 ± 0.07	2.95 ± 0.14	3.00 ± 0.14
0.5–1 mm	T_0	CTRL	9.30 ± 0.10	9.34 ± 0.11	9.31 ± 0.06	1.89 ± 0.03	1.97 ± 0.04	2.00 ± 0.05
	T_1	FM + M2	17.52 ± 0.89	18.94 ± 1.16	19.68 ± 1.10	3.57 ± 0.08	3.62 ± 0.16	3.70 ± 0.13
	T_2	FM + M19	16.84 ± 0.58	18.19 ± 0.74	18.91 ± 0.61	3.60 ± 0.07	3.65 ± 0.26	3.74 ± 0.24
	T_3	FM + M22	18.52 ± 0.71	19.98 ± 0.52	20.79 ± 0.77	3.46 ± 0.04	3.50 ± 0.21	3.58 ± 0.19
	T_4	PM + M2	16.83 ± 0.17	18.43 ± 0.27	19.16 ± 0.34	3.53 ± 0.06	3.57 ± 0.14	3.66 ± 0.11
	T_5	PM + M19	16.36 ± 0.46	18.36 ± 0.32	19.09 ± 0.16	3.54 ± 0.06	3.59 ± 0.23	3.67 ± 0.20
	T_6	PM + M22	17.51 ± 0.48	18.45 ± 0.72	19.18 ± 0.58	3.57 ± 0.05	3.62 ± 0.17	3.70 ± 0.15
	T_7	MO + M2	17.52 ± 0.45	18.46 ± 0.53	19.19 ± 0.52	3.26 ± 0.08	3.31 ± 0.27	3.38 ± 0.25
	T_8	MO + M19	17.46 ± 0.30	18.39 ± 0.28	19.12 ± 0.29	3.35 ± 0.07	3.40 ± 0.25	3.47 ± 0.22
	T_9	MO + M22	17.82 ± 0.19	18.76 ± 0.16	19.51 ± 0.15	3.21 ± 0.08	3.26 ± 0.25	3.33 ± 0.23

Table 4. *Cont.*

Aggregate Size (mm)	Treatments	Organic + Microbe	Water Stable Aggregates (%)			Aggregate Organic Carbon (g kg^{-1})		
			Days after Incubation			Days after Incubation		
			14th	28th	42nd	14th	28th	42nd
0.25–0.5 mm	T_0	CTRL	18.48 ± 0.37	18.64 ± 0.26	18.96 ± 0.11	2.17 ± 0.04	2.30 ± 0.04	2.35 ± 0.09
	T_1	FM + M2	28.60 ± 0.43	29.65 ± 0.72	30.71 ± 1.35	3.33 ± 0.07	3.39 ± 0.25	3.46 ± 0.23
	T_2	FM + M19	28.22 ± 0.40	29.27 ± 0.89	30.29 ± 1.24	3.23 ± 0.10	3.27 ± 0.19	3.35 ± 0.17
	T_3	FM + M22	29.53 ± 0.49	30.62 ± 0.81	31.71 ± 1.44	3.25 ± 0.09	3.30 ± 0.22	3.37 ± 0.20
	T_4	PM + M2	29.49 ± 0.47	30.59 ± 0.99	31.64 ± 1.05	3.30 ± 0.07	3.35 ± 0.24	3.43 ± 0.22
	T_5	PM + M19	29.00 ± 0.29	30.05 ± 0.21	31.10 ± 0.84	3.21 ± 0.12	3.25 ± 0.18	3.32 ± 0.17
	T_6	PM + M22	29.27 ± 0.66	30.37 ± 1.20	31.43 ± 1.44	3.27 ± 0.12	3.31 ± 0.14	3.38 ± 0.12
	T_7	MO + M2	26.45 ± 0.55	27.44 ± 1.03	28.40 ± 1.25	3.26 ± 0.08	3.32 ± 0.26	3.39 ± 0.24
	T_8	MO + M19	25.24 ± 0.32	26.17 ± 0.66	27.06 ± 0.63	3.21 ± 0.11	3.26 ± 0.28	3.33 ± 0.26
	T_9	MO + M22	26.10 ± 0.33	27.04 ± 0.17	27.99 ± 0.86	3.20 ± 0.10	3.25 ± 0.27	3.32 ± 0.25
0.106–0.25 mm	T_0	CTRL	33.10 ± 0.88	32.78 ± 1.85	32.03 ± 1.31	2.96 ± 0.07	2.99 ± 0.21	3.06 ± 0.19
	T_1	FM + M2	24.66 ± 0.57	24.34 ± 0.86	23.52 ± 0.31	2.73 ± 0.15	2.78 ± 0.27	2.84 ± 0.25
	T_2	FM + M19	28.08 ± 0.98	25.43 ± 0.57	24.08 ± 0.26	2.52 ± 0.05	2.55 ± 0.12	2.61 ± 0.10
	T_3	FM + M22	25.73 ± 1.05	24.15 ± 0.27	23.63 ± 0.25	2.74 ± 0.11	2.78 ± 0.25	2.84 ± 0.23
	T_4	PM + M2	25.07 ± 1.00	24.30 ± 0.43	23.78 ± 0.46	2.74 ± 0.12	2.77 ± 0.07	2.83 ± 0.05
	T_5	PM + M19	26.43 ± 0.65	26.61 ± 0.76	25.60 ± 0.65	2.54 ± 0.08	2.58 ± 0.22	2.64 ± 0.21
	T_6	PM + M22	26.30 ± 0.82	24.59 ± 0.34	24.08 ± 0.27	2.76 ± 0.08	2.79 ± 0.12	2.85 ± 0.10
	T_7	MO + M2	26.60 ± 0.83	24.95 ± 1.55	23.11 ± 0.28	2.85 ± 0.09	2.90 ± 0.24	2.96 ± 0.23
	T_8	MO + M19	26.74 ± 1.03	26.24 ± 0.61	25.26 ± 0.83	2.69 ± 0.06	2.72 ± 0.11	2.78 ± 0.10
	T_9	MO + M22	25.88 ± 0.87	25.56 ± 0.64	24.89 ± 0.85	2.83 ± 0.09	2.86 ± 0.08	2.92 ± 0.07
<0.106 mm	T_0	CTRL	34.59 ± 1.39	34.87 ± 1.49	35.69 ± 1.83	3.80 ± 0.12	3.71 ± 0.21	3.41 ± 0.25
	T_1	FM + M2	11.24 ± 0.24	11.09 ± 0.32	9.44 ± 0.54	2.19 ± 0.08	2.35 ± 0.05	2.48 ± 0.04
	T_2	FM + M19	14.58 ± 0.41	13.89 ± 0.52	13.05 ± 0.14	2.23 ± 0.09	2.39 ± 0.07	2.52 ± 0.08
	T_3	FM + M22	13.10 ± 0.59	12.16 ± 0.51	11.34 ± 0.62	2.16 ± 0.12	2.32 ± 0.08	2.45 ± 0.07
	T_4	PM + M2	13.28 ± 0.43	11.92 ± 0.56	11.11 ± 0.36	2.05 ± 0.11	2.20 ± 0.13	2.31 ± 0.14
	T_5	PM + M19	15.27 ± 0.60	11.93 ± 0.54	10.66 ± 0.63	2.27 ± 0.06	2.44 ± 0.04	2.57 ± 0.05
	T_6	PM + M22	15.27 ± 0.77	12.60 ± 0.31	10.77 ± 0.51	2.27 ± 0.10	2.44 ± 0.13	2.57 ± 0.15
	T_7	MO + M2	15.45 ± 0.77	14.76 ± 0.84	14.17 ± 0.74	2.53 ± 0.12	2.72 ± 0.10	2.87 ± 0.10
	T_8	MO + M19	16.62 ± 0.33	15.19 ± 0.32	14.68 ± 0.35	2.51 ± 0.10	2.69 ± 0.14	2.83 ± 0.15
	T_9	MO + M22	16.31 ± 0.86	14.61 ± 0.32	13.17 ± 0.46	2.59 ± 0.12	2.79 ± 0.15	2.93 ± 0.17

Variation in the number of total organics of soil throughout the experiment is accessible from Figure 2 approving authenticity of carbon receptivity with an artificial application of organics along with EPS-producing rhizobacteria. Figure 2 explicates the retention and degradation of artificially added organic materials with the stretch of incubation duration. Samples collected on the 14th day of the study enfolded 11.21 g kg^{-1} organics content in T_1 that was statistically in line with farm manure and poultry treated soils under all the three strains but were significantly greater than the molasses treated soils and control unit. Organic carbon was reduced to 11.03 and 10.82 g kg^{-1} under T_2, which was statistically similar to farm manure-treated soils but significantly higher than poultry and molasses, whose degradation is much faster under such circumstances.

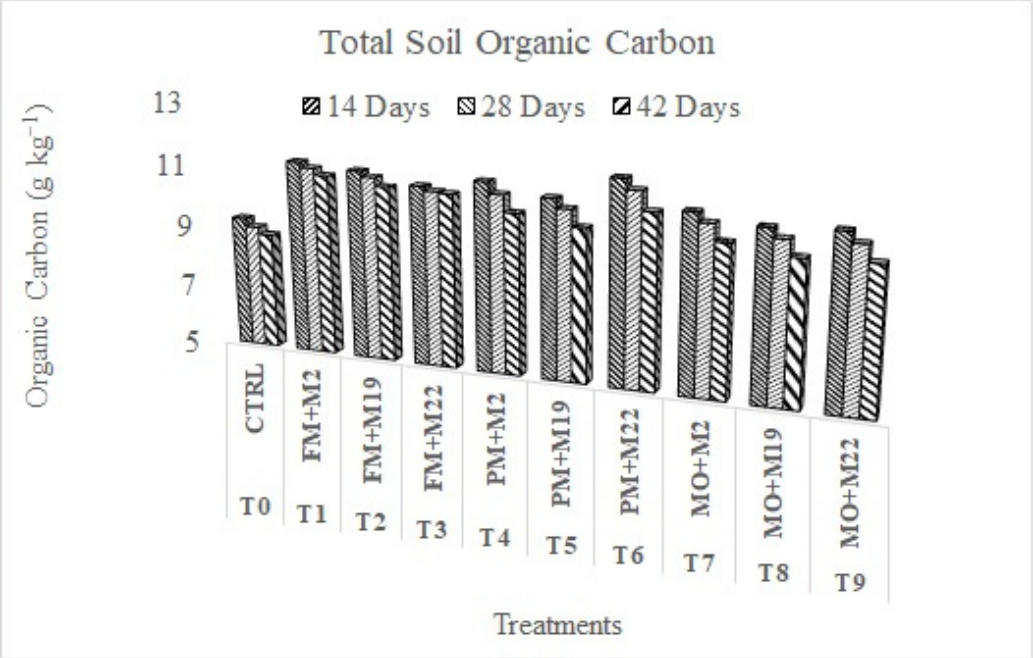

Figure 2. Variation in soil total organic carbon content with time passage upon the blended application of organic substrates and bacterial strains.

Cumulative respiration from soil was unexpectedly high in molasses-treated soils than in control and other manures (Figure 3), possibly explained by CO_2 released due to continued microbial stabilization. In the present study, organo-microbially treated soils had noticeably greater labile organic fractions compared with control treatments (Figures 4 and 5). Artificially added organics, as well as rhizobacterial inputs, provided more carbon compared with the untreated soil. Hence, a substantial increase in labile fraction with applied organo-microbial treatments shifts the dynamics of carbon relative to the control treatment.

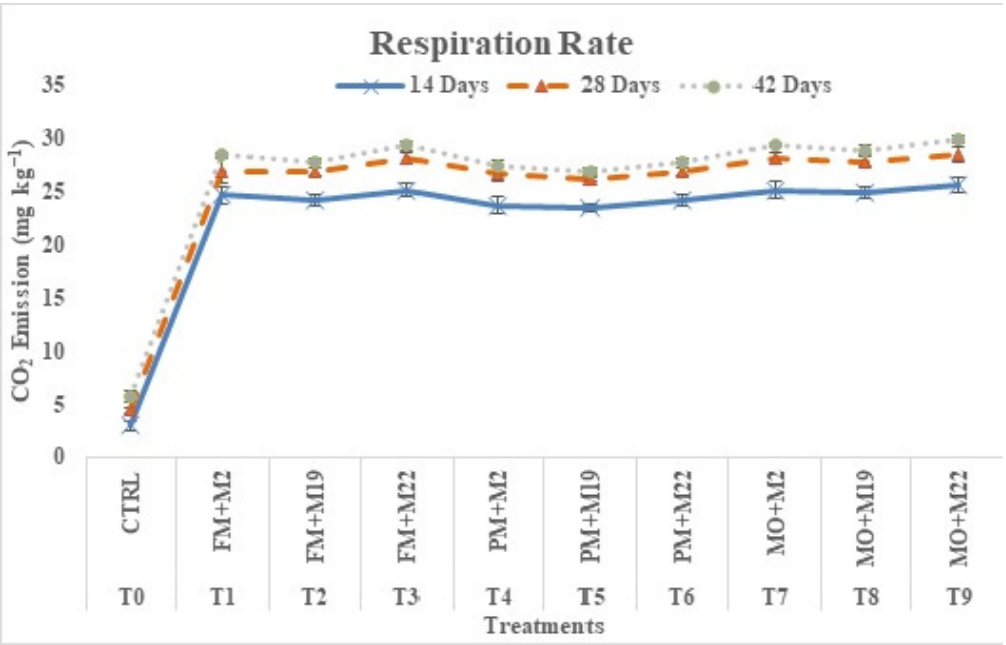

Figure 3. Variation in microbial respiration with time passage upon blended application of organic substrates and bacterial strains.

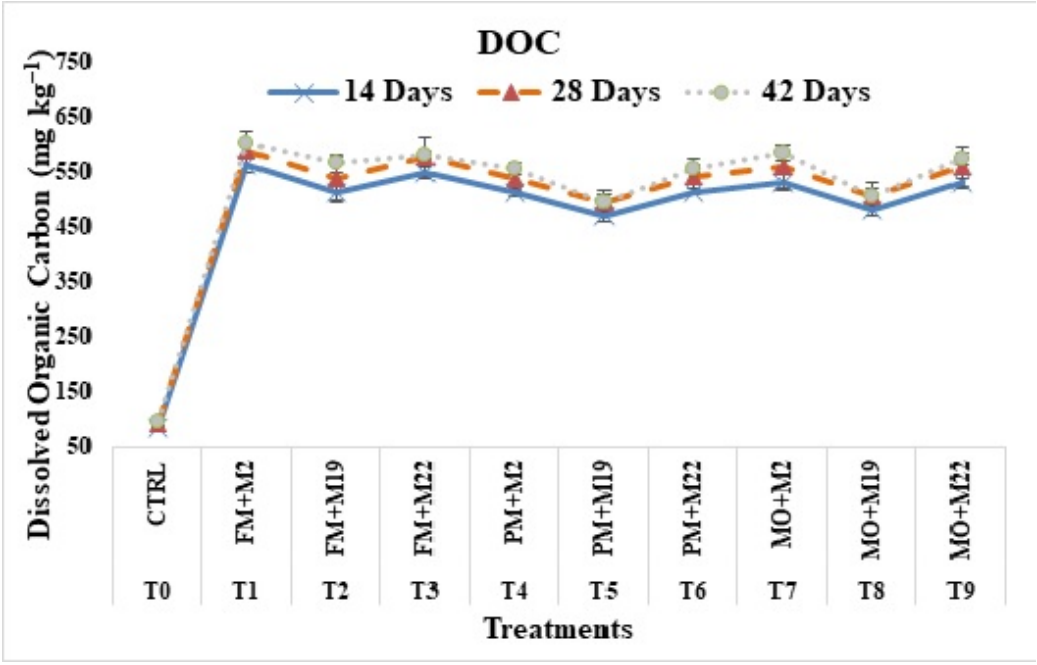

Figure 4. Variation in dissolved soil organic carbon content with time passage upon blended application of organic substrates and bacterial strains.

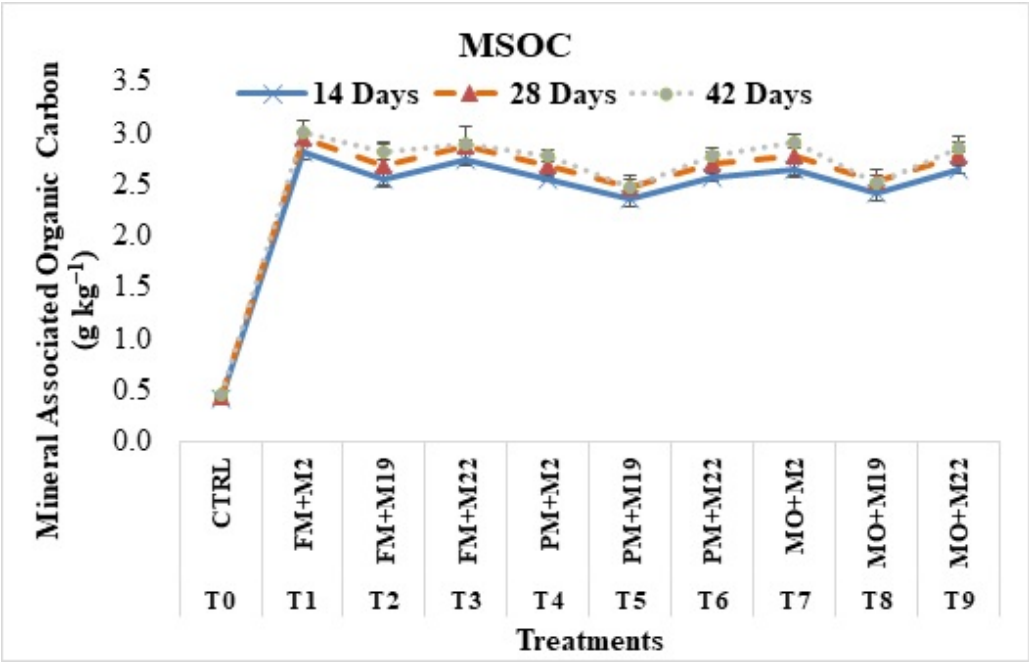

Figure 5. Variation in mineral-associated organic carbon with time passage upon blended application of organic substrates and bacterial strains.

The amount of water retained in the soil during the experiment upon the application of organic materials and rhizobacterial strains is expounded in Table 5. On the 14th day of incubation treatment, T_2 retained 24.14% water at 0.33 MPa suction, which was statistically similar with farm manure and poultry treated soils in the presence of each strain but had a significant difference from control and molasses treated units. Water retention was enhanced with time passage and a similar trend of variation was observed on the 28th and 42nd days of the experiment, with T2 retaining 12.6 and 16% more water at field capacity level than the control. Hygroscopic contents of water varied from 11.54, 12.01, and 11.85%

in T2 to 10.64, 11.04, and 11% in the control on respective days of sample collection with no significant difference. Water held in meso and micropores was increased from 11.91, 11.16, and 11.11% in the control to 12.6, 13, and 13.80% in T2 at each sample collection time. T2 had significantly greater available water content than the control but was at par with all other treatments.

Table 5. Variation in water retention characteristics with manure and microbial blends.

	Organic + Microbe	Treatments	Days after Incubation		
			14th	28th	42nd
FC	CTRL	T_0	22.55 ± 0.87	22.20 ± 0.53	22.12 ± 0.53
	FM + M2	T_1	24.14 ± 0.35	25.01 ± 0.26	25.65 ± 0.23
	FM + M19	T_2	23.52 ± 0.30	23.77 ± 0.89	24.30 ± 1.12
	FM + M22	T_3	23.60 ± 0.37	23.73 ± 0.37	23.87 ± 0.80
	PM + M2	T_4	23.97 ± 0.58	24.19 ± 0.42	24.23 ± 0.21
	PM + M19	T_5	23.39 ± 0.26	23.45 ± 0.76	23.93 ± 1.02
	PM + M22	T_6	23.87 ± 0.11	24.12 ± 0.83	24.20 ± 1.30
	MO + M2	T_7	22.91 ± 0.18	23.14 ± 0.63	23.33 ± 1.04
	MO + M19	T_8	23.19 ± 0.16	23.48 ± 0.73	23.73 ± 1.11
	MO + M22	T_9	23.23 ± 0.62	23.36 ± 0.81	22.89 ± 1.24
PWP	CTRL	T_0	10.64 ± 0.41	11.04 ± 0.26	11.00 ± 0.26
	FM + M2	T_1	11.54 ± 0.18	12.01 ± 0.27	11.85 ± 0.15
	FM + M19	T_2	11.10 ± 0.14	11.59 ± 0.25	11.59 ± 0.13
	FM + M22	T_3	11.13 ± 0.18	11.55 ± 0.35	11.33 ± 0.21
	PM + M2	T_4	11.31 ± 0.27	11.82 ± 0.41	11.53 ± 0.52
	PM + M19	T_5	11.03 ± 0.12	11.44 ± 0.22	11.35 ± 0.12
	PM + M22	T_6	11.26 ± 0.05	11.67 ± 0.13	11.37 ± 0.12
	MO + M2	T_7	10.81 ± 0.08	11.21 ± 0.19	10.99 ± 0.09
	MO + M19	T_8	10.94 ± 0.07	11.41 ± 0.18	11.25 ± 0.10
	MO + M22	T_9	10.96 ± 0.29	11.39 ± 0.40	10.84 ± 0.10
AWC	CTRL	T_0	11.91 ± 0.46	11.16 ± 0.27	11.11 ± 0.27
	FM + M2	T_1	12.60 ± 0.20	13.00 ± 0.03	13.80 ± 0.12
	FM + M19	T_2	12.43 ± 0.16	12.18 ± 0.99	12.71 ± 1.19
	FM + M22	T_3	12.47 ± 0.20	12.18 ± 0.71	12.54 ± 0.96
	PM + M2	T_4	12.67 ± 0.30	12.37 ± 0.73	12.70 ± 0.59
	PM + M19	T_5	12.36 ± 0.14	12.02 ± 0.92	12.58 ± 1.09
	PM + M22	T_6	12.61 ± 0.06	12.44 ± 0.94	12.82 ± 1.21
	MO + M2	T_7	12.10 ± 0.09	11.92 ± 0.82	12.34 ± 1.05
	MO + M19	T_8	12.25 ± 0.09	12.06 ± 0.89	12.49 ± 1.14
	MO + M22	T_9	12.27 ± 0.33	11.97 ± 0.86	12.05 ± 1.19

5. Discussion

Intricacy makes soil the most challenging environment to work with, so additional methodologies for the understanding of soil are used [45,46]. Our approach is to intricate the soil aggregation and carbon retention upon the artificial application of organics and rhizobacterial strains. Our study assesses the dominance of macro aggregates in farm manure and poultry in the presence of all strains. Organic scums are microbial triggering

catalysts that induce particle binding to formulate macro aggregates [3]. Increased organic matter in organics amended soils favored macro aggregation, mounting confrontation to slaking. Other studies have correspondingly testified noteworthy escalation in mean weight diameters (MWD) [47,48]. Greater macro aggregate extents have been found to favor soil structural stabilization, which might be an upshot of an increase in soil cementers, i.e., rhizobacterial EPS exudation [49]. Organic manures are comprised of saccharides, aliphatic, and aromatic amalgams that are a source of energy and nutrition for soil microbes and plant roots that produce EPS [15]. Bacterial and fungal debris bind the primary (sand, silt, and clay) particles to extremely stable micro aggregates, while transient (plant and microbe derived EPS) and temporary (hyphae, roots, and bacterial cells) binders formulate macro aggregates, minimizing carbon putrefaction because of physical protection through sorption to clay minerals and encapsulation within aggregates [50]. Mycorrhizal fungi produce microbial glue, and proteoglycan "Glomalin" to formulate and stabilize macro aggregates [51]. In our study, manure application improved the microhabitat of microbes, facilitating rhizobacterial growth, density, and effectiveness [52]. Fungal hyphae physically bind the particles together to enhance aggregate stability [3].

Organic cementers (rhizobacterial exudates (EPS)) amass primary particles and micro aggregates to yield macro aggregates with greater carbon contents according to the aggregate hierarchy conceptual model [3]. In the interim, macro aggregates afford soil organics protection mechanisms [24,53]. Soiled manure heightened macro aggregate protected carbon accumulation [54] is supposed to be an imperative practical approach to increase structural stability and sequestration of carbon [55,56]. Physical protection is one of the most important tools for SOC equilibrium and its degree of recalcitrance depends upon its position in aggregates [57].

The active soil carbon fraction that changes quickly is microbial biomass carbon (MBC) [22,58]. Soil microbes largely depend upon the spatial distribution of carbon in the soil to which soil microorganisms are most sensitive. Aggregates are ecological niches having heterogeneously distributed microorganisms in various aggregate fractions [59].

Cumulative respiration from soil was unexpectedly high in molasses-treated soils than in control and other manures (Figure 3), possibly explained by CO_2 released due to continued microbial stabilization [60]. Highly variable respiration rates were observed [61] in similar soils with variable soil physiognomies and incubation conditions. It is a speculated elucidation that molasses may easily be putrefied to create differences in soil respiration compared with control and other organics. Decaying the behavior of organics in soil fluctuates depending upon the substance and soil type as the microbial activity is regulated by the substrate's molecular complexity and soil factors, i.e., soil pH and nutritious status [62,63].

Larger-sized aggregates possessed more SOC than smaller ones (Table 4), which is consistent with the aforementioned findings in other soils. Bronick and Lal [49] found greater SOC contents in smaller-sized aggregates, but more recent findings from Jiang et al. [54] are heavier amounts of SOC in macro aggregates. Macro aggregate-associated SOC may rapidly be stabilized and decomposed due to larger size [64], as micro aggregates are strongly bound. Labile (dissolved and mineral-associated) organic carbon fractions play a conclusive role in aggregate formation and stabilization [13]. In the present study, organo-microbially treated soils had noticeably greater labile organic fractions compared with control treatments (Figures 4 and 5). It's possibly due to the greater amount of organics inputs associated with rhizobacterial strains, as has been observed by Rudrappa et al. [65]. Artificially added organics, as well as rhizobacterial inputs, provided more carbon compared with untreated soil. Hence, a substantial increase in labile fraction with applied organo-microbial treatments shifts the dynamics of carbon relative to the control treatment.

SOC greatly contributes to aggregation, which accounts for 70–90% approximated variation in aggregate stability of clay loam soil. Total SOC is vital for particle aggregation, more specifically labile fractions are directly involved in aggregation [56]. These findings are consistent with our results presented in Figure 6.

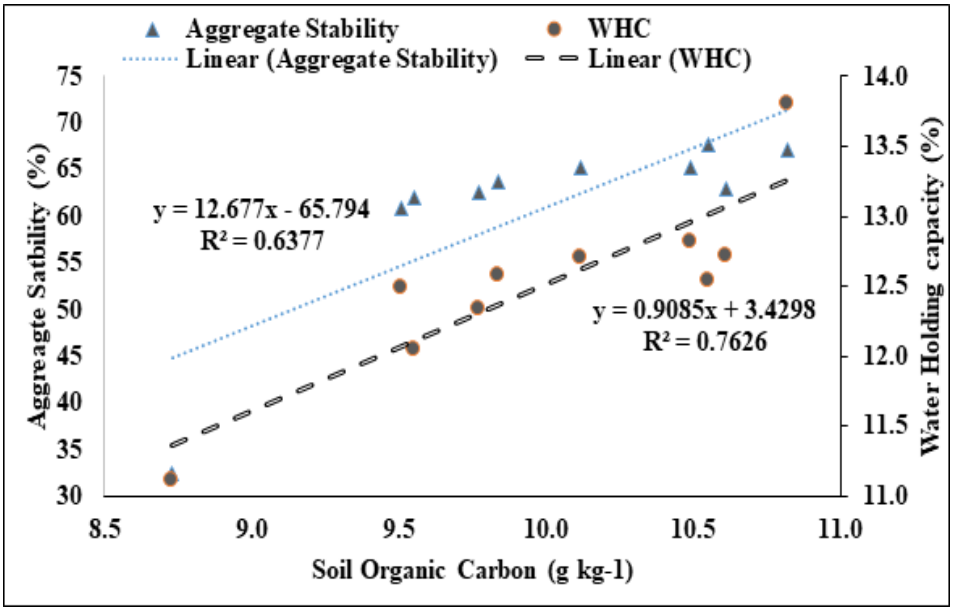

Figure 6. Variation of water stable aggregation and water retention capacities of soil with the time passage.

Low water stable aggregates in desert soils might be due to low SOC [5], while the greatest magnitudes of total SOC and carbohydrates were yielded by soils with the highest aggregate stability [20]. Efficient acceleration of crusting in aggregates greater than 0.25 mm in size, declining water and soil losses [6]. Thus, organics application gives rise to macro aggregates, improving soil structure, and restoring water to create a supportive environment for plant growth. Applied organics retain water and additionally supply water-soluble, hydrolysable organic substrates, leading to the production of microbial exopolymers that increase aggregate cohesion ultimately increasing water stable aggregates having excessive pores to retain more water [23]. In this study, it was elaborated that microbe amendment blends improve soil structure but more effectively four blends (FM + M_2, FM + M_{22}, PM + M_{19}, MO + M_{19}) are categorized best through clustering of the data through cluster analysis (Figure 7).

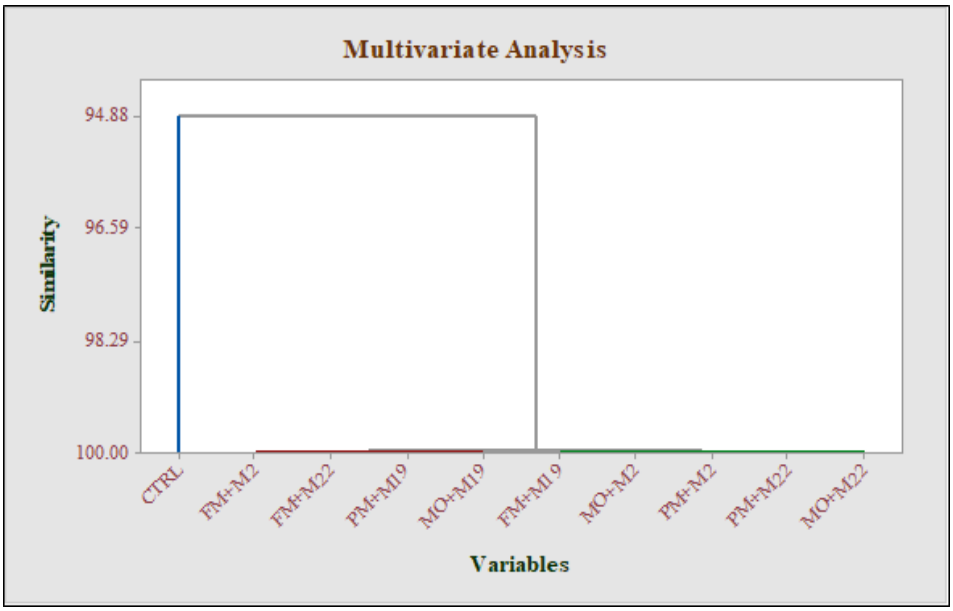

Figure 7. Multivariate analysis to select better-performing treatments.

6. Conclusions

Compared to naturally present inorganic agents, the short-term application of organics (farm manure, poultry, and molasses) combined with rhizobacterial strains improved soil structure to different extents by regulating soil aggregate distribution and stability. Meanwhile, soil labile and aggregate-associated carbon proportion rather than the total amount of soil carbon are suggestively enhanced with the combined application of manures and rhizobacterial strains. The contents of total SOC gradually reduced over time, probably due to microbial degradation but the extent of degradation varied depending upon manure type and applied microbe. Overall, amalgamated application of organic manures and EPS-producing microbes might be most effective technique for soil structural stabilization and soil organic carbon sequestration under sandy clay loam texture. A long-term comprehensive evaluation is necessary to verify the most suitable combination for improving soil quality and organic carbon sequestration in sandy clay loam soil under an arid climate.

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