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Impact of Integrated Nutrient Sources on Soil Biological Health: Assessing Microbial Biomass Carbon and Population Shifts in Cauliflower (Brassica oleracea var. botrytis) Rhizosphere

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ABSTRACT

Soil microbial communities are fundamental indicators of soil health and fertility, directly influencing nutrient cycling and plant productivity in sustainable agriculture. This study investigated the comparative effects of three distinct nutrient amendments—triple superphosphate (a mineral phosphate fertilizer), a phosphate-solubilizing biofertilizer, and vermicompost—on key biological parameters in soil cultivated with cauliflower (Brassica oleracea var. botrytis). The parameters assessed included soil microbial biomass carbon (SMBC), a robust measure of active microbial biomass, and the concomitant changes in culturable bacterial and fungal population densities. A pot or field experiment was conducted using a randomized complete block design with [X] treatments and [Y] replicates. Soil samples were collected at critical growth stages (e.g., seedling, curd initiation, and harvest) and analyzed using established protocols: the chloroform fumigation-extraction method for SMBC and serial dilution plating on selective media for bacterial and fungal counts. Preliminary results indicate significant treatment-based variations. Vermicompost application consistently promoted the highest SMBC and fostered a more abundant and diverse microbial community, likely due to its stable organic matter and nutrient complexity. The biofertilizer treatment also showed a significant enhancement in SMBC and specific bacterial populations compared to the unamended control, highlighting its role in mobilizing indigenous nutrients. In contrast, the triple superphosphate treatment, while effective in plant phosphorus nutrition, resulted in a less pronounced or moderated effect on overall microbial biomass and composition. These findings underscore the critical role of organic and bio-based amendments in enhancing soil biological quality, which is integral to developing sustainable nutrient management strategies for Brassica vegetable production systems within the agri-food industry.

1. INTRODUCTION

Soil biomass is the portion of organic matter that is involved with the soil. It's considered to be an important indicator of soil fertility, and is associated with the destruction of organic matter and the conversion of nutrients.Any alterations to biomass activity will have a biological effect on processes and interactions in the soil, which in turn will have an effect on plant growth.[1] In general, soil biomass contains up to 5% organic carbon and nitrogen. These elements are released when organisms die. Biomass has two important functions: first, oxidation of nitrogen- and carbon-containing substances and second, storage of the contents of these elements and minerals in living cells. [2] Soil biomass contributes to the disintegration of organic substances. and is considered an indicator of physical and chemical changes caused by soil management processes and environmental factors, which affect nutrient availability by increasing plant readiness. The activity of soil biomass can be assessed by the amount of nitrogen and carbon consumed or taken up by microbial cells, as these are among the most important elements that microorganisms use as a source of energy for their vital activities.[3] Several investigations have demonstrated that the activity of soil The biomassotal bacterial and fungal counts is affected by the soil type and the conditions in which it is cultivated. , and increased biomass activity is observed when fertilizers are added. Mineral fertilizers, organic fertilizers, and bio fertilizers increase the concentration of carbon, nitrogen, and phosphorus in the soil, which is necessary for the life of microorganisms in the soil. as a result, the purpose of this study. was to study the alterations in biomass carbon With total bacterial and fungal counts after adding TSP fertilizer, biofertilizer, and vermicompost during the Flowering and Full Maturity Stages of cauliflower.

2. Materials and Methods

A field experiment was conducted during the growing season at one of the agricultural farms located in Al-Daghara city, Al-Diwaniyah Governorate, Iraq. The experimental soil exhibited specific chemical, physical, and biological properties, as presented in Table 1. The study aimed to evaluate the effects of triple superphosphate (TSP), biofertilizer (*Bacillus subtilis*), and vermicompost on microbial biomass carbon and total bacterial and fungal populations in soil planted with cauliflower during the flowering and full maturity stages.

The experiment was arranged according to a randomized complete block design (RCBD) with three replications. Each experimental unit consisted of four planting rows, with a total of 20 plants per unit. Triple superphosphate was applied at three levels: 0, 50, and 100 kg ha^{-1}, designated as P0, P1, and P2, respectively. Biofertilizer treatments included non-application (B0) and application (B1). Vermicompost was applied at three levels: 0, 10, and 20 t ha^{-1}, designated as V0, V1, and V2, respectively.

Microbial biomass carbon was determined using the chloroform fumigation–extraction (CFE) method, as described by [5]. Total bacterial and fungal populations were estimated using standard plate count techniques following the method reported by [9].

Table 1. Some of the chemical, physical and biological properties of the study soil before planting.

Character	Value	Unit	Source
pH $_{1:1}$	7.81	----	[6]
EC $_{1:1}$	2.86	ds m^{-1}	
Organic matter	7.33	g kg^{-1}soil	
Ready phosphor	10.21		[7]
Ready potassium	153.36		
Ready nitrogen	23.17	mg kg^{-1}soil	

texture Soil	mixture		[8]
Apparent density	1.43	mg m^{-3}	
Total bacteria	18.4×10^6		[9]
Total fungi	4.6×10^3	CFU g^{-1} dry soil	

Table 2. Some chemical and physical properties of vermicompost.

Character	**Value**	**Unit**
Moisture	25	%
pH	7.6	----------
N	1.69	
P_2O_5	0.0103	%
K_2O	0.394	
O.C	20	%
O.M	32.77	
C/N	15-25	----------
Zn	27-40	
Cu	50-90	
Mn	15-25	ppm
Fe	26-50	

3. Results and Discussion

3.1. Microbial Biomass Carbon (MBC) at Flowering and Full Maturity Stages

The results presented in Tables 3 and 4 and illustrated in Figure 1 indicate that the application of biofertilizer (B1) had a significant effect on microbial biomass carbon (MBC) during both the flowering and full maturity stages. The highest MBC values under biofertilizer treatment reached 216.48 and 178.00 mg C kg^{-1} at the flowering and full maturity stages, respectively. In contrast, the lowest MBC values were recorded under the non-biofertilized treatment (B0) at both stages. The increase in microbial biomass carbon following biofertilizer application can be attributed to enhanced microbial respiration and the accelerated decomposition of organic substrates, which stimulate biological activity in the soil [9].

Vermicompost application also resulted in significant changes in MBC during both growth stages. The highest MBC values were observed under the V2 treatment, while the lowest values occurred in the control treatment (V0). Recorded values reached 232.35 mg C kg^{-1} at the flowering stage and 185.48 mg C kg^{-1} at the full maturity stage. The positive effect of vermicompost is likely due to its contribution to soil organic carbon, which serves as a readily available energy source for soil microorganisms, thereby promoting microbial growth and activity.

Similarly, the addition of triple superphosphate (TSP) significantly influenced microbial biomass carbon at both developmental stages. The highest MBC values associated with TSP application reached 215.06 mg C kg^{-1}, whereas the control treatment (P0) recorded the lowest value of 135.23 mg C kg^{-1}. These results suggest that phosphorus availability plays a critical role in enhancing microbial metabolic processes and biomass accumulation.

Significant interactions were observed between biofertilizer and vermicompost treatments. The combined application of B1V2 resulted in the highest MBC values at both growth stages, reaching 245.32 and 194.50 mg C kg^{-1}, respectively, while the B0V0 treatment showed the lowest values. This interaction highlights the synergistic effect of organic amendments and beneficial microorganisms in improving soil biological properties.

The interaction between biofertilizer and TSP also significantly affected MBC. The B1P2 combination produced the highest MBC values at both stages (234.15 and 186.80 mg C kg^{-1}), whereas the lowest values were associated with the control treatment (B0P0), particularly during the flowering stage. These findings indicate that the combined supply of phosphorus and microbial inoculation enhances microbial efficiency and biomass formation.

In addition, the interaction between vermicompost and TSP significantly influenced microbial biomass carbon, with notable differences observed between treatments. The highest mean MBC value (252.99 mg C kg^{-1}) was recorded under the highest fertilization levels, while the lowest values occurred in the control treatments.

The three-way interaction among TSP, biofertilizer, and vermicompost had a highly significant effect on MBC at both growth stages. Compared with the control treatment (B0V0P0), the combined application of B1V2P2 resulted in the highest microbial biomass carbon values, reaching 264.13 mg C kg^{-1} at the flowering stage and 202.67 mg C kg^{-1} at the full maturity stage. These results are consistent with previous studies reporting that integrated use of mineral fertilizers, organic amendments, and biofertilizers enhances soil microbial biomass and activity [11,12].

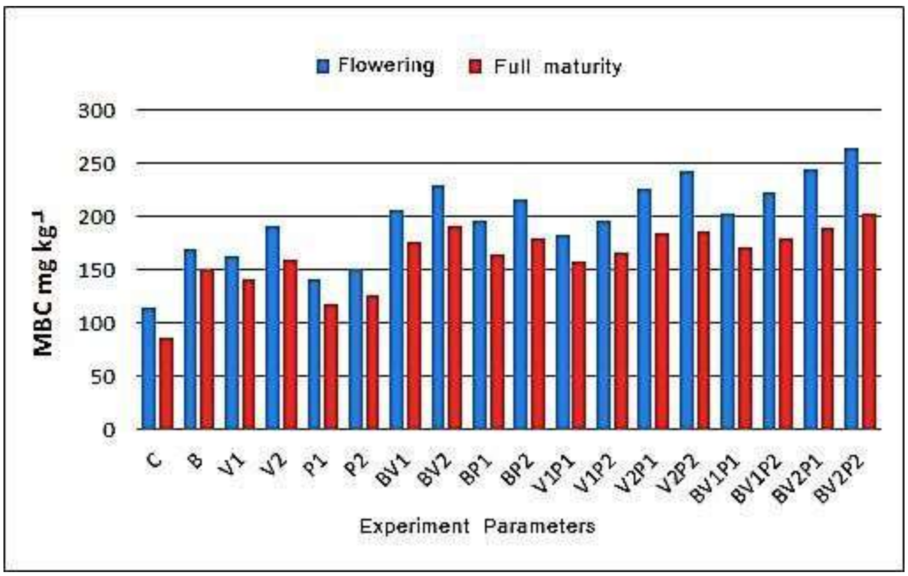

Figure 1. Effects of triple superphosphate (TSP), biofertilizer, and vermicompost, individually and in combination, on microbial biomass carbon (MBC) of cauliflower rhizosphere soil during the flowering and full maturity stages.

Table 3. Effects of triple superphosphate (TSP), biofertilizer, and vermicompost, individually and in combination, on microbial biomass carbon (MBC) of cauliflower rhizosphere soil at the flowering stage.

Biofertilizer	B0		B1
	178.26		216.48
LSD 0.05		2.05	
Vermicompost	V0	V1	V2
	164.37	195.38	232.35
LSD 0.05		2.51	
TSP	P0	P1	P2
	178.65	198.38	215.06
LSD 0.05		2.51	
B ×V Interactions			
	V0	V1	V2

B0		135.20	180.18	219.38
B1		193.54	210.57	245.32
LSD 0.05			3.56	
		B ×P Interactions		
		P0	P1	P2
B0		155.86	182.94	195.97
B1		201.45	213.83	234.15
LSD 0.05			3.56	
		V ×P Interactions		
		P0	P1	P2
V0		142.25	168.06	182.80
V1		184.26	192.48	209.39
V2		209.46	234.60	252.99
LSD 0.05			4.36	
		B ×V × P Interactions		
		P0	P1	P2
	V0	114.46	141.02	150.12
B0	V1	162.55	182.06	195.94
	V2	190.56	225.73	241.85
	V0	170.03	195.11	215.48
B1	V1	205.96	202.89	222.85
	V2	228.35	243.48	264.13
LSD 0.05			6.16	

Table 4. Effects of triple superphosphate (TSP), biofertilizer, and vermicompost, individually and in combination, on microbial biomass carbon (MBC) of cauliflower rhizosphere soil at the full maturity stage.

Biofertilizer	B0		B1
	147.19		178.00
LSD 0.05		1.59	
Vermicompost	V0	V1	V2
	137.19	165.10	185.48
LSD 0.05		1.94	
TSP	P0	P1	P2
	150.53	164.11	173.15
LSD 0.05		1.94	
	B ×V Interactions		
	V0	V1	V2
B0	110.18	154.91	176.47
B1	164.21	175.29	194.50
LSD 0.05		2.75	
	B ×P Interactions		
	P0	P1	P2
B0	128.92	153.14	159.50
B1	172.13	175.07	186.80
LSD 0.05		2.75	
	V ×P Interactions		
	P0	P1	P2
V0	117.93	140.73	152.92
V1	158.42	164.92	171.96
V2	175.23	186.66	194.56
LSD 0.05		3.37	

B ×V × P Interactions				
		P0	P1	P2
B0	V0	86.49	117.42	126.61
	V1	141.11	158.2	165.43
	V2	159.17	183.8	186.45
B1	V0	149.37	164.04	179.24
	V1	175.74	171.65	178.48
	V2	191.29	189.53	202.67
LSD 0.05			4.76	

3.2 Bacterial Count in Soil at Flowering and Full Maturity Stages .

The results presented in Tables 5 and 6 indicate that biofertilizer application significantly increased total bacterial counts in the soil at both the flowering and full maturity stages. The B1 treatment (biofertilizer addition) recorded the highest bacterial populations, reaching 63.96 and 48.19 CFU g^{-1} dry soil $\times 10^6$, respectively, whereas the control treatment (B0) exhibited the lowest values, with 50.90 and 33.34 CFU g^{-1} dry soil $\times 10^6$ at the two stages. This enhancement is attributed to the capacity of biofertilizers to naturally stimulate bacterial growth and population density in the rhizosphere [13].

Similarly, vermicompost application had a significant effect on total bacterial counts during both stages. The highest bacterial populations were observed under the V2 treatment, with values of 66.47 and 48.97 CFU g^{-1} dry soil $\times 10^6$ at the flowering and full maturity stages, respectively. In contrast, the control treatment (V0) showed the lowest counts, with 50.32 and 32.32 CFU g^{-1} dry soil $\times 10^6$. The increase in bacterial abundance following vermicompost application is likely due to the provision of additional organic matter, which supplies essential nutrients and serves as a carbon and energy source for microbial metabolism and proliferation [14].

Furthermore, the application of phosphate fertilizer (TSP) significantly influenced bacterial populations. The P2 treatment exhibited the highest counts at both stages, with 61.75 and 45.41 CFU g^{-1} dry soil $\times 10^6$, whereas the control (P0) had the lowest values, 53.43 and 35.60 CFU g^{-1} dry soil $\times 10^6$. This increase is attributable to the essential role of phosphorus in microbial growth and metabolic processes, supporting cell structure, energy transfer, and enzymatic activity [15].

Overall, these findings demonstrate that individual and combined applications of biofertilizer, vermicompost, and phosphate fertilizers enhance soil bacterial populations, thereby improving soil microbial health and nutrient cycling.

Table 5. Effects of biofertilizer, vermicompost, and triple superphosphate (TSP) on bacterial counts (CFU g^{-1} dry soil $\times 10^6$) in cauliflower rhizosphere soil at the flowering stage.

Biofertilizer	B0		B1
	50.90		63.96
LSD 0.05		1.26	
Vermicompost	V0	V1	V2
	50.32	55.50	66.47
LSD 0.05		1.54	
TSP	P0	P1	P2
	53.43	57.12	61.75
LSD 0.05		1.54	
	B × V Interactions		
	V0	V1	V2

B0		42.50	49.19	61.01
B1		58.14	61.81	71.93
LSD 0.05			2.18	
			B × P Interactions	
		P0	P1	P2
B0		46.77	50.60	55.33
B1		60.10	63.63	68.16
LSD 0.05			N.S	
			V × P Interactions	
		P0	P1	P2
V0		47.14	49.97	53.85
V1		51.02	54.91	60.57
V2		62.13	66.47	70.82
LSD 0.05			N.S	
			B × V × P Interactions	
		P0	P1	P2
B0	V0	40.82	41.05	45.62
	V1	42.79	48.44	56.33
	V2	56.68	62.32	64.04
B1	V0	53.46	58.90	62.08
	V1	59.25	61.38	64.81
	V2	67.59	70.62	77.60
LSD 0.05			3.77	

Table ٦. Effect of biofertilizer, vermicompost and TSP on bacterial counts in soil (CFU g-1 dry soil $\times10^{٦}$) at the fully mature stage .

Biofertilizer	B0 33.34		B1 48.19
LSD 0.05		1.25	
Vermicompost	V0 32.32	V1 41.00	V2 48.97
LSD 0.05		1.53	
TSP	P0 35.60	P1 41.28	P2 45.41
LSD 0.05		1.53	
		B × V Interactions	
	V0	V1	V2
B0	25.16	32.52	42.35
B1	39.48	49.48	55.60
LSD 0.05		N.S	

		P0	P1	P2
P × B Interactions				
		P0	P1	P2
B0		28.10	33.60	38.33
B1		43.10	48.96	52.50
LSD 0.05			N.S	
P × V Interactions				
		P0	P1	P2
V0		28.64	31.97	36.35
V1		35.02	41.91	46.07
V2		43.13	49.97	53.82
LSD 0.05			N.S	
P × V × B Interactions				
		P0	P1	P2
B0	V0	21.82	24.05	29.61
	V1	24.79	33.44	39.33
	V2	37.68	43.32	46.04
B1	V0	35.46	39.90	43.08
	V1	45.25	50.38	52.81
	V2	48.59	56.62	61.60
LSD 0.05			N.S	

The two-way interaction between biofertilizer and vermicompost significantly affected total bacterial counts at the flowering stage. The combination of B1V2 produced the highest bacterial population, reaching 71.93 CFU g^{-1} dry soil $\times 10^6$, while the control treatment (B0V0) recorded the lowest value of 42.50 CFU g^{-1} dry soil $\times 10^6$. However, this interaction did not result in significant differences during the full maturity stage. The two-way interactions between biofertilizer and phosphate fertilizer, as well as between vermicompost and phosphate fertilizer, showed no significant effects on bacterial counts at either the flowering or full maturity stages.

In contrast, the three-way interaction among biofertilizer, vermicompost, and phosphate fertilizer significantly influenced bacterial populations at the flowering stage. The combination B1V2P2 yielded the highest bacterial count of 77.60 CFU g^{-1} dry soil $\times 10^6$, whereas the control (B0V0P0) had the lowest count of 40.82 CFU g^{-1} dry soil $\times 10^6$. This result was not significantly different from intermediate treatments such as B0V0P1 and B0V1P0. At the full maturity stage, however, no significant differences were observed due to these interactions.

These findings can be explained by the sensitivity of bacterial populations—particularly Actinobacteria, which constitute a substantial fraction of rhizosphere bacteria—to nutrient availability, especially nitrogen and phosphorus [16]. In addition, the presence of organic matter improves soil physical properties, enhances fertility and moisture retention, and stimulates the release of root exudates and growth regulators, including organic acids such as lactic and ascorbic acids. These conditions promote bacterial motility and facilitate their colonization of the root surface and surrounding rhizosphere [17].

3.3. Fungal Count in Soil at Flowering and Full Maturity Stages

The results presented in Tables 7 and 8 show that biofertilizer application significantly increased total fungal counts in the soil during both the flowering and full maturity stages. The B1 treatment (biofertilizer addition) produced the highest fungal populations, with 29.91 and 21.57 CFU g^{-1} dry soil $\times 10^3$ at the two stages, respectively, compared to the control treatment (B0), which recorded the lowest values of 24.99 and 16.59 CFU g^{-1} dry soil $\times 10^3$. This enhancement is attributed to the presence of *Bacillus subtilis* in the biofertilizer, which stimulates root exudation and promotes symbiotic interactions between fungi and the host plant, thereby supporting fungal proliferation in the rhizosphere [18].

Vermicompost application also significantly affected total fungal counts. The V2 treatment recorded the highest values, 32.38 and 24.10 CFU g^{-1} dry soil $\times 10^3$, while the control treatment (V0) showed the lowest counts, 22.55 and 13.96 CFU g^{-1} dry soil $\times 10^3$. The increase in fungal populations is likely due to microbial mineralization of organic matter, which releases essential nutrients such as carbon, nitrogen, and phosphorus. Furthermore, decomposed organic matter can slightly acidify the soil and enhance nutrient availability, creating favorable conditions for fungal growth. Since fungi are heterotrophic organisms, they actively participate in organic matter decomposition, explaining their increased abundance under higher vermicompost levels [19].

Phosphate fertilizer (TSP) application also led to significant increases in total fungal counts at both growth stages. The P2 treatment yielded the highest values, 29.96 and 21.82 CFU g^{-1} dry soil $\times 10^3$, whereas the control (P0) recorded the lowest counts, 24.70 and 16.41 CFU g^{-1} dry soil $\times 10^3$. This effect can be attributed to enhanced root development and exudation induced by phosphorus, which creates a conducive environment for fungal growth. Additionally, phosphorus stimulates ribosomal RNA (rRNA) synthesis and chitin production, an essential structural component of fungal cell walls [20].

Significant two-way interactions were observed for biofertilizer × vermicompost. The combination B1V2 produced the highest fungal counts at both stages, 35.95 and 28.13 CFU g^{-1} dry soil $\times 10^3$, compared to the control B0V0, which showed the lowest values, 20.89 and 12.62 CFU g^{-1} dry soil $\times 10^3$. Similarly, the biofertilizer × phosphate fertilizer interaction significantly increased fungal counts, with the B1P2 treatment reaching 32.98 and 25.07 CFU g^{-1} dry soil $\times 10^3$, whereas the B0P0 control had the lowest counts, 23.50 and 15.42 CFU g^{-1} dry soil $\times 10^3$. These differences, however, were not significantly different from intermediate treatments such as B0P1.

No significant effects were observed for the vermicompost × phosphate fertilizer two-way interaction or the three-way interaction among biofertilizer, vermicompost, and phosphate fertilizer at either growth stage. Nevertheless, the application of vermicompost and phosphate fertilizers appears to enhance fungal growth indirectly by promoting root biomass and increasing the secretion of sugars and organic acids, which attract microbes and facilitate symbiotic interactions with plant cells [21].

Table 7. Effects of biofertilizer, vermicompost, and triple superphosphate (TSP) on fungal counts (CFU g^{-1} dry soil $\times 10^3$) in cauliflower rhizosphere soil at the flowering stage.

Biofertilizer	B0		B1
	24.99		29.91
LSD 0.05		1.20	
Vermicompost	V0	V1	V2
	22.55	27.41	32.38
LSD 0.05		1.48	
TSP	P0	P1	P2
	24.70	27.68	29.96
LSD 0.05		1.48	
B × V Interactions			
	V0	V1	V2
B0	20.89	25.26	28.81
B1	24.22	29.56	35.95
LSD 0.05		2.09	
P × B Interactions			
	P0	P1	P2
B0	23.50	24.52	26.94
B1	25.91	30.84	32.98
LSD 0.05		2.09	
P × V Interactions			

		P0	P1	P2
V0		20.76	22.43	24.48
V1		24.57	27.82	29.83
V2		28.77	32.79	35.58
LSD 0.05			N.S	
P × V × B Interactions				
		P0	P1	P2
B0	V0	19.97	19.79	22.92
	V1	24.01	24.94	26.84
	V2	26.51	28.84	31.07
B1	V0	21.56	25.07	26.03
	V1	25.14	30.71	32.83
	V2	31.03	36.75	40.09
LSD 0.05			N.S	

Table 8. Effects of biofertilizer, vermicompost, and triple superphosphate (TSP) on fungal counts (CFU g^{-1} dry soil $\times 10^3$) in cauliflower rhizosphere soil at the full maturity stage.

Biofertilizer		B0		B1
		16.59		21.57
LSD 0.05			1.40	
Vermicompost		V0	V1	V2
		13.96	19.19	24.10
LSD 0.05			1.71	
TSP		P0	P1	P2
		16.41	19.02	21.82
LSD 0.05			1.71	
B × V Interactions				
		V0	V1	V2
B0		12.62	17.09	20.08
B1		15.29	21.29	28.13
LSD 0.05			2.42	
Interactions B × P				
		P0	P1	P2
B0		15.42	15.78	18.58
B1		17.39	22.25	25.07
LSD 0.05			2.42	
Interactions V × P				
		P0	P1	P2
V0		12.59	13.81	15.47
V1		16.29	19.25	22.02
V2		20.34	23.99	27.98
LSD 0.05			N.S	
B Interactions × V × P				
		P0	P1	P2
B0	V0	12.02	11.91	13.93
	V1	15.97	16.03	19.25
	V2	18.27	19.41	22.55
B1	V0	13.16	15.71	17.02
	V1	16.61	22.48	24.78

V2	22.41	28.57	33.41
LSD 0.05		N.S	

4.Conclusions

The study demonstrated that the application of biofertilizer, vermicompost, and triple superphosphate significantly enhanced microbial biomass carbon (MBC) as well as total bacterial and fungal populations in cauliflower rhizosphere soil at both the flowering and full maturity stages. The lowest MBC and microbial counts were observed at the full maturity stage, likely due to reduced microbial activity and decreased root growth.

These findings indicate that the addition of organic, mineral, and biofertilizers increases carbon content in microbial biomass through multiple mechanisms: by directly stimulating microbial activity, by promoting root development and exudation that provide additional substrates for microorganisms, and by contributing organic carbon directly to the soil. Overall, fertilization strategies combining organic and mineral amendments can enhance soil microbial activity, nutrient cycling, and soil fertility, supporting improved plant growth and productivity.

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