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Field Evaluations *of Rhizobium tropici* Extracellular Polymeric Substances on Soil Fertility Indices, Plant Growth, and Development

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Abstract

Extracellular polymeric substances (EPS) derived from microorganisms have been shown to improve soil physical properties and used for soil erosion control. Due to their ability to enhance plant nutrient uptake, it was postulated that they could serve as a valuable natural resource to improve agricultural productivity. Black-eyed peas (BEPs) were planted in a field experiment on a Decatur silt loam soil amended with *Rhizobium tropici*-derived EPS, and their effects were evaluated on certain soil fertility indices, plant growth, and development potentials. The EPS increased soil CO_2 flux and microbial biomass compared to the control (unamended soil). Soil microbial population shifted towards Gram-negative bacteria at the initial stages of applications and plant growth, and subsequently towards Gram-positive bacteria in the amended soils. The active carbon, total soil N, soil nitrate, and available phosphorus were higher in soils amended with EPS compared to the control. The applied EPS increased several plant growth parameters, including stem height, branches per plant, leaf area index, root length, leaves per plant, and leaf chlorophyll content. Plants' seed yields increased by 22% compared to the control, while the shoot biomass and root nodules counts per plant increased by 38.1% and 51.5%, respectively.

Keywords

Extracellular Polymeric Substances (EPS), Black-Eyed Peas (BEPs), *Rhizobium tropici*, Plant Growth, Seed Yield, Microbial Biomass

1. Introduction

The use of excretory-secretory products derived from microorganisms as a valuable natural resource to improve agricultural productivity has been extensively highlighted [1]. Microbially-derived biopolymers are considered alternatives to conventional chemical polymers due to their ease of biodegradability, non-toxicity, and renewability [2]. Extracellular polymeric substances (EPS) from bacteria have been shown to enhance soil aggregate stability, improve plant nutrient uptake, bind metal cations, and mitigate plant salt stresses [3]. *Rhizobium tropici* is a legume symbiont known for its high capacity to produce biodegradable and environmentally friendly EPS [4] at a low production cost [5].

Changes in soil health indices can be evaluated by monitoring the appropriate indicators over time to assess the effectiveness of a farming system, land use practices, or new technologies aimed at improving crop production [6]. While inherent soil health attributes may show limited or no changes over time, dynamic soil fertility attributes are affected by current soil management practices [7] [8]. Soil respiration and microbial biomass are critical in assessing soil life during short or prolonged periods of soil perturbation. Studies on the effects of soil amendments on soil fertility factors have been conducted in long-term agricultural experimental setups spanning numerous seasons [9] [10], which may overlook short-term changes in soil biological indices.

The studies reported have focused on their effects on soil physical indicators, soil erosion control, and plant growth [11]-[13]. In this study, we quantitatively assessed the effects of Black-eyed pea growth and *R. tropici* EPS on changes in soil biological properties. The specific objectives were (a) to quantify short-term soil CO_2 flux and soil microbial biomass changes in soils amended with *R. tropici* EPS and planted with Black-eyed peas, (b) to understand how a combination of soil amendment with EPS and the growth of Black-eyed peas affect soil life and (c) to evaluate the effects of amending soils with EPS on crop yield.

Black-eyed peas plant (*Vigna unguiculata*) is a popular protein-rich legume cover crop used as a food source at various stages of its development and eaten as green or dried beans. It is so named because of the prominent black spot on the seeds at the point where they attach to the pod. As a cover crop, it has been utilized to enhance water retention and capture and store carbon, thereby mitigating global climate change [14] [15]. Because of its rapid growth, the Black-eyed pea is a suitable plant for short-term field studies.

2. Materials and Methods

2.1. Study Site, Experimental Design, EPS Applications, and Seeding

The study site was at the Winfred Thomas Agricultural Research Station (WTARS), Alabama A&M University (GPS coordinates: 34.90136˚ N, 86.55971˚ W (WGS84)). The soil is classified as Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults) and has been hay-fallowed for over 5 years. The usual climate is humid

and subtropical with hot summers and relatively mild winters, with occasional cold days and temperatures below the freezing point. The mean daily air temperature and precipitation recorded from June to August 2023 were 24.6˚C and 3.9 mm, respectively.

The Black-eyed peas (BEPs) (*Vigna unguiculata*) used were obtained from C. T Garvin Seeds and Feeds, Huntsville, Alabama. The land was mowed and plowed before applying the EPS solution. The experimental design with 3 blocks and 4 plots per block was a Randomized Complete Block Design (RCBD) with an experimental size of 16 × 30 m. The first plot (Plot 1) was amended with EPS but not planted with BEP. The second plot (Plot 2) had EPS and Black-eyed peas, the third plot (Plot 3) had Black-eyed peas only, while the fourth plot (Plot 4), or control, was without EPS and Black-eyed peas. The block size was 16 × 10 m, while the plot size was 4 × 10 m. In three split applications, *R. tropici* EPS solution (10 g/L) was added to the soil at 0.1% (w/w) at 10 cm depth. An equivalent volume of tap water was added to the control plot without EPS. The amount of 0.1% EPS was selected because in a previous greenhouse experiment, when the Decatur silt loam soil was amended with 0%. 0.02%, 0.1%, and 0.5% EPS (w/w), microbial biomass, and root biomass were significantly reduced at 0.5% compared to the control, whereas 0.1% EPS increased shoot and root biomass as well as microbial biomass.

A rake and pike roller were used to spread the EPS on the plots, and later, a Honda FRC800 soil roller tiller was used to mix the EPS with the topsoil. The seeds were planted 3 days after the initial EPS application and allowed to grow under free rain without irrigation throughout the experiment. Seed germination was monitored each day for 12 days, during which the weeds were removed from all the plots manually and without herbicide applications.

2.2. Soil Chemical Analysis

Soil samples were collected from four different locations in each plot at 0 - 15 cm depth to form a composite sample for the treatment and analyzed for soil pH, soil organic matter (SOM) [16], exchangeable bases, cation exchange capacity (CEC), nitrate [17], available phosphate [18], total nitrogen (TN) [19], and permanganate oxidizable carbon (POx-C) [20]. Each analysis was in triplicate.

2.3. Soil CO_2 Flux, Soil Temperature, Moisture, and Electrical Conductivity

Soil CO_2 flux was measured from each of the plots on days 4, 8, 13, 16, 20, 23, 27, 30, 34, 37, 41, 48, 51, 58, and 65 after EPS application at areas where polyvinyl chloride collars (20 cm in diameter, 11.4 cm in height) were inserted into the soil. The collars were at fixed positions in each plot for the duration of the experiment and distributed to represent the various sections of the plot.

To take soil CO_2 flux measurements, a semi-automated chamber (8200-01S, LiCor Inc., Lincoln, NE, USA) was situated tightly on top of the pre-placed collars. The chamber was paired with an infrared analyzer, the LI-8100 soil CO_2 flux sys-

tem (LiCor Inc., Lincoln, NE, USA), to monitor CO_2 concentration inside the chamber. SoilFluxProTM (LiCor Inc., Lincoln, NE, USA) was used to calculate soil CO_2 flux based on the exponential increase in CO_2 concentration. There were 3 collars per plot, and two measurements were done for each collar, giving a total of 6 readings per plot, the average of which was reported. All flux measurements were taken from approximately 9 a.m. to noon to minimize inter-day variation by the diurnal pattern of soil CO_2 flux. Soil temperature, soil moisture, and electrical conductivity were measured in the field along with CO_2 flux measurements using the Em50 Series Data Collection System (Decagon Devices, Inc., USA) inserted into the soil at 5 cm depth, within 20 cm away from the collars.

2.4. Soil Microbial Biomass

Soil microbial biomass and changes in community composition were estimated using the phospholipid fatty acid (PLFA) analysis technique. Phospholipids were extracted using 0.5 g freeze-dried soil samples and methylated with methanolic KOH to form fatty acid methyl esters (FAMEs) [21] and then analyzed with an Agilent Technologies DB-5 ms column 7890B gas chromatograph (Agilent Technologies, CA, USA). The soil microbial groups were identified based on some PLFAs, which are either unique to or distinctly more common in specific taxonomic groups [22]-[25]. The following PLFAs were used as taxonomic biomarkers: The content of C18:2ω6,9 for fungal biomass, C16:1ω5 for the biomass of *arbuscular mycorrhizal* fungi (AMF), C18:1ω7, C17:1ω8, and cy17:0 for biomass of Gram-negative bacteria (GNB), the iso and anteiso branched-chain fatty acids, i15:0, i17:0, and a15:0 for biomass of Gram-positive bacteria (GPB), 10Me16:0, 10Me17:0, and 10Me18:0 for biomass of Actinobacteria (Actino). The biomass ratios of Gram-positive to Gram-negative bacteria (GPB/GNB) and fungi to bacteria (F/B) were used to determine the changes in microbial composition in soils [26]. The ratio of monounsaturated fatty acids to cyclopropane fatty acids (*i.e.*, 16:1ω7c + 18:1ω7c)/(cy17:0 + cy19:0) was used to measure Gram-negative (GN) stress in soils [27].

2.5. Plant Growth and Seed Yield

Plant performance was assessed by measuring the number of leaves per plant, leaf area index (LAI), leaf chlorophyll content, and stem height. The leaf area index was measured using the AccuPAR LP-80 Ceptometer (Decagon Devices, METER Group, Inc., USA). The leaf chlorophyll content was assessed using the SPAD-502 digital chlorophyll meter (Spectrum Technologies, Plainfield, Ill.) [28].

At the flowering stage, thirty-five (35) days after germination, 10 plants from each plot were randomly selected and carefully uprooted from the soil. Root lengths, number of root nodules per plant, shoot, and root fresh weight were recorded for plants grown on amended and unamended soils. After data collection of fresh weight, dry weight was obtained by placing the plant material in an oven at 105˚C for 1 h to inhibit the enzymatic oxidation of polyphenolic compounds

and to accelerate the evaporation of foliar water.

At plant maturity, 33 plants from each plot were randomly selected and assessed for the number of branches per plant, number of pods per plant, seeds per pod, weight of dry pod, and seed weight. The plant seed yield per hectare was estimated as follows:

Yield/ha = pods/plant × seeds/pod × weight/seed × plants/ha.

2.6. Statistical Analysis

The effects of EPS on the growth and yield parameters of Black-eyed peas on EPS-amended and unamended soils (control) were analyzed using two-sample t-tests. The data for soil chemical properties, CO_2 flux, and soil microbial biomass were analyzed by analysis of variance (ANOVA) test and Tukey's test to assess the significance of the differences between treatments using SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). A single-factor analysis of variance (ANOVA) was conducted to determine whether amending soils with EPS and or planting with Black-eyed peas significantly affected soil CO_2 flux. An effect was considered significant at a p-value less than 0.05. The correlations between soil CO_2 flux, soil temperature, and moisture were estimated by Pearson correlation, using the SAS 9.4 software package. Correlation was regarded as weak (between 0.1 - 0.3), moderate (between 0.3 - 0.7), and strong (between 0.7 - 1.0).

3. Results

3.1. Soil Chemical Properties

The results of the soil chemical properties are shown in Tables 1a and 1b. Six days after applying EPS, the soil pH, soil organic matter (SOM), available P, total nitrogen (TN), and permanganate oxidizable carbon (POx-C) increased compared to the reference soils (control), though not significantly (Table 1a). After forty-nine days, soil nitrate was considerably higher in EPS-amended soils than in the control (Table 1b). Although available phosphorus, total nitrogen, and active carbon were also higher in the EPS-amended soils, the increase was not significant. Soil nitrate was higher in EPS-amended soils than in the amended soils with Black-eyed peas (BEPs). Soil total nitrogen was higher in soils amended with EPS, but increased with BEPs planted in the amended soils.

Table 1a. Soil chemical properties six (6) days after applying EPS.

Treatment	pH	SOM (LOI %)	CEC (meq/100 g)	NO_3^--N	P	Total N	POx-C
				ppm			
EPS	6.8[a]	4.7[a]	9.0[b]	28.3[a]	42.0[a]	2082[a]	807.7[a]
EPS + BEP	6.8[a]	4.5[a]	8.8[b]	23.7[a]	35.0[a]	1822[a]	756.3[a]
BEP	6.5[a]	4.4[a]	10.8[a]	26.5[a]	34.3[a]	1777[a]	777.7[a]
Control	6.4[a]	4.4[a]	10.8[a]	34.0[a]	35.0[a]	1785[a]	781.0[a]

Values (means for each parameter) with similar letters indicate not statistically different based on Tukey's Studentized Range (HSD) comparison with $\alpha = 0.05$.

Table 1b. Soil chemical properties forty-nine (49) days after applied EPS.

Treatment	pH	SOM (LOI %)	CEC (meq/100 g)	NO_3^--N	P	Total N	POx-C
				ppm			
EPS	6.1[a]	4.6[a]	11.7[a]	64.1[a]	29.3[a]	1812[a]	653.3[a]
EPS + BEP	6.5[a]	4.5[a]	10.1[a]	21.0[b]c	29.3[a]	1855[a]	724.0[a]
BEP	6.3[a]	4.5[a]	11.9[a]	16.1c	28.7[a]	1793[a]	720.7[a]
Control	6.0[a]	4.4[a]	10.8[a]	35.3[b]	27.3[a]	1792[a]	611.3[a]

Values (means for each parameter) with similar letters indicate not statistically different based on Tukey's Studentized Range (HSD) comparison with $\alpha = 0.05$.

3.2. Soil CO_2 Flux Changes

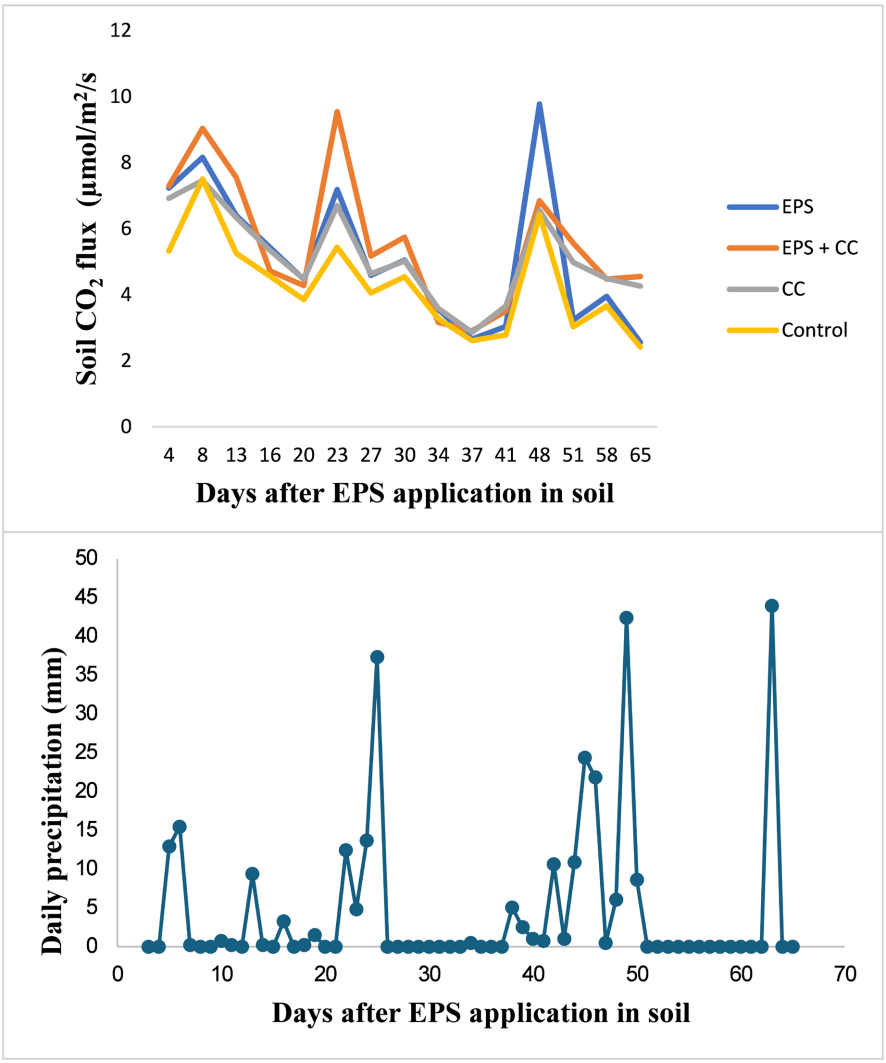


Figure 1. Variations in soil CO_2 flux and rainfall at the study site. For the CO_2 flux graph, the blue line represents soils with EPS, the pink line represents soil amended with EPS and planted with BEP cover crop (CC), the grey line represents soils with the cover crop but no EPS, and the orange line is the control.

Soil CO_2 flux varied among measurement days, ranging from 2.56 - 9.77 $\mu molm^{-2}s^{-1}$ (EPS-amended soils), 2.9 - 9.54 $\mu molm^{-2}s^{-1}$ (EPS-amended soils planted with BEP), 2.86 - 7.46 $\mu molm^{-2}s^{-1}$ (unamended soils with BEP), and 2.42 - 7.5 $\mu molm^{-2}s^{-1}$ (unamended soil without BEP). The mean CO_2 flux values (in $\mu mol/m^2/s$) for EPS-amended soil, unamended soil grown with BEP, amended soil grown with BEP, and unamended soil without BEP (control) were 5.15, 5.15, 5.62, and 4.31, respectively. Although the CO_2 flux was higher for soils amended with EPS or in combination with BEPs a one-way ANOVA revealed that there was no statistically significant difference in the mean flux values of the different soils ($F(3, 14) = 32.9$, $p = 0.06$). However, the day of measurement appeared to have affected the CO_2 flux. The lowest values were observed for the unamended soils (control), while the highest values were obtained for EPS-amended soils or the amended soils planted to BEP, depending on the time of the measurements. The results showed that on the 8th and 23rd day after EPS application, the CO_2 flux of EPS-amended soils planted with BEP peaked much higher than the control (Figure 1). However, on day 48, the CO_2 flux of the soils amended with EPS and without BEP peaked much higher compared to the control. These peaks in the CO_2 flux corresponded to the peaks in daily precipitation at the study site (Figure 1).

The results of soil temperature measurements indicated that EPS-amended soils had a higher soil temperature than the unamended soils. Soil temperature in degrees Celsius varied with time of measurements, ranging from 33.5 to 38.4 (EPS-amended soils), 31.4 to 37.1 (EPS-amended soils with BEP cover crops), 32.2 to 37.5 (unamended soils with BEPs), and 31.9 to 37.5 (control). The lowest soil temperature was recorded for EPS-amended soils grown to BEP, while the highest temperature was recorded for EPS-amended soils. The growth of cover crops reduced the soil temperature. After 58 days of applied EPS, the lowest temperatures were recorded for amended soils grown with BEP.

Table 2. Pearson correlation between soil CO_2 flux and soil moisture content (SMC), soil temperature (ST), and soil electrical conductivity (EC) for soils amended with EPS and/or planted with Black-eyed pea cover crop (CC).

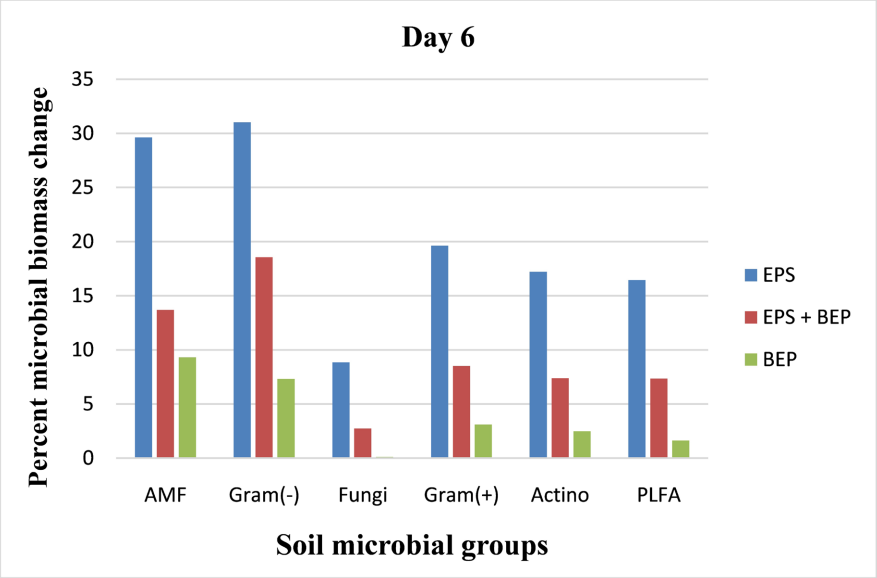
	CO_2 flux			
	EPS	CC	EPS + CC	Control
ST	0.231	0.061	0.002	0.345
SMC	0.430*	0.644*	0.523*	0.255
EC	0.571*	0.789*	0.683*	0.611*

*Correlation is significant at the 0.05 level.

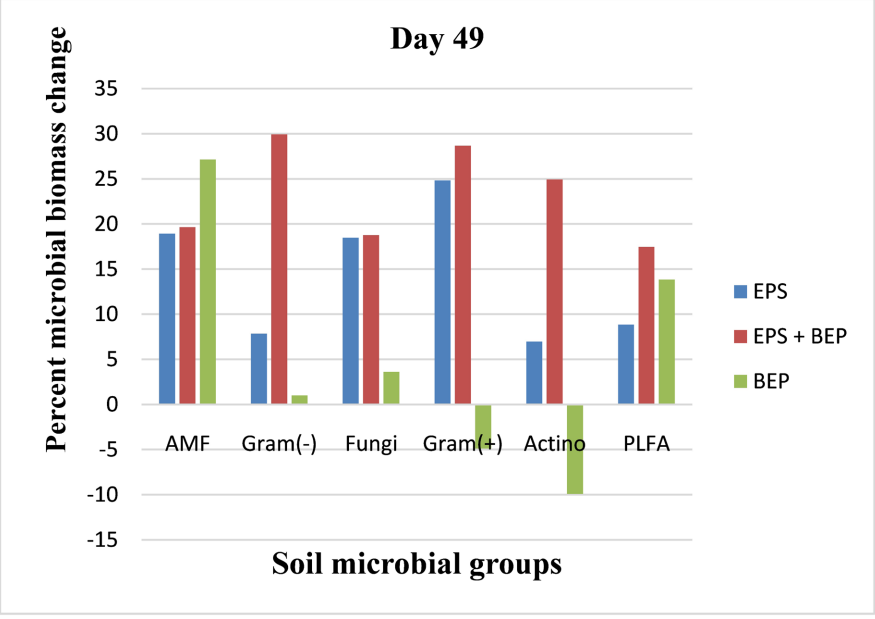
Soil CO_2 flux showed a weak correlation with soil temperature (ST) and a moderate correlation with soil moisture and electrical conductivity (EC) (Table 2). Generally, the moisture content of the EPS-amended soil was higher than that of the control (unamended soil). The growth of cover crops on EPS-amended soils further increased soil moisture. Results showed that when the soils were amended

with *R. tropici* EPS and planted to BEP cover crop, soil CO_2 flux became less dependent on soil temperature ($r = 0.002$, $p < 0.01$) and more dependent on soil moisture ($r = 0.523$, $p < 0.05$). A weak positive correlation was observed between soil CO_2 flux and ST ($r = 0.231$, $p < 0.05$) when the soil was amended with EPS without BEPs.

3.3. Soil Microbial Biomass



(a)



(b)

Figure 2 Changes in total soil microbial biomass (PLFA) and microbial groups in soils amended with EPS or planted with Black-eyed pea (BEP), (a) six days after applying EPS; (b) forty-nine days after applying EPS.

Results showed that EPS alone and in combination with BEP growth increased the total microbial biomass (PLFA) and that of most microbial groups compared to the control (No EPS and no BEP). Applying the biopolymer to soils increased *Arbuscular mycorrhizal* fungi (AMF) and Gram-negative (GN) bacteria biomass by over 25% compared to the control within the first week of application (Figure 2(a)). Applying only EPS increased the biomass of most microbial groups than when the EPS-amended soil was planted with BEPs. After forty-nine days of applied biopolymer, EPS favored the growth of Gram-positive bacteria (GPB). In contrast, the growth of BEP on EPS-amended soils favored the growth of Gram-negative bacteria (GNB). However, the growth of BEP on unamended soils increased the growth of AMF more than the amended soils (Figure 2(b)). The study showed that growing cover crops on EPS-amended soils significantly increased the biomass of all microbial groups than growing on unamended soils.

The shift in the soil microbial community structure was estimated by the microbial biomass ratios of the various microbial groups (Table 3). From the analysis of microbial biomass ratios (GPB/GNB and F/B), results showed that one week after the application of EPS to soil, both EPS and BEP growth favored Gram-negative bacteria (GNB) proliferation. After forty-nine days, EPS favored the growth of Gram-positive bacteria (GPB), while BEP favored the growth of GNB. The EPS and BEP growth increased the ratio of the fungi to bacterial biomass. The results also showed that EPS significantly increased the ratio of monounsaturated fatty acids to cyclopropane fatty acids (16:1Ω7c + 18:1Ω7c)/(cy17:0 + cy19:0) measured as the Gram-negative (GN) stress (Table 3), where the higher the value, the lower the environmental stress.

Table 3. Soil microbial biomass ratios.

Soil Treatment	Soil Microbial Biomass Ratios				
	6 days after EPS application		49 days after EPS application		
	GPB/GNB	F/B	GPB/GNB	F/B	GN stress
Control	1.80^{a}	0.45^{a}	0.74^{b}	1.89^{c}	0.63^{d}
EPS	1.64^{d}	0.39^{d}	0.83^{a}	1.92^{b}	0.67^{a}
EPS + BEP	1.65^{c}	0.41^{c}	0.72^{c}	1.73^{d}	0.66^{b}
BEP	1.73^{b}	0.43^{b}	0.67^{d}	2.02^{a}	0.65^{c}

Mean values with a similar letter indicate not significantly different based on Tukey's Studentized Range (HSD) test at $\alpha = 0.05$.

3.4. Seed Germination, Plant Growth, and Seed Yield

The number of seeds germinated was monitored each day for 12 days for soils with or without EPS amendments. Applied EPS seemed to have increased the germination rate of BEPs. A germination rate of 75.4% was recorded for soils amended with EPS and 71% for unamended soils. The percentage of BEP seeds germinated with time is shown in Figure 3.

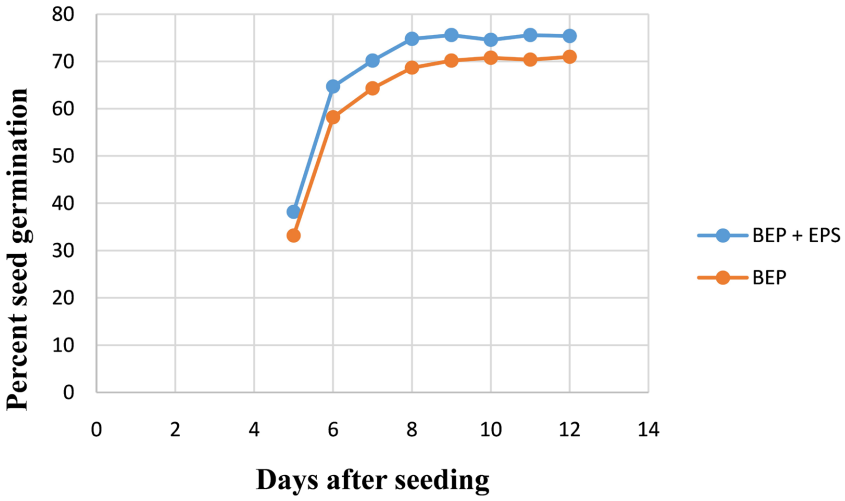


Figure 3. Germination rate of Black-eyed pea seeds in soils with or without EPS amendments.

A two-sample t-test was performed to compare plant growth parameters between Black-eyed peas (BEPs) grown on EPS-amended soils and those grown on unamended soils. There was a significant difference in the average number of root nodules between BEPs grown on EPS-amended soils (M = 25.3, SD = 10.0) and those grown on unamended soils (M = 16.7, SD = 7.3); t (28) = 2.70, p = .011). Although the average stem height (SH), branches per plant (BPP), leaves per plant (LPP), leaf area index (LAI), leaf chlorophyll content (LCC), shoot dry mass (SDM) and root dry mass (RDM) were higher for plants grown on EPS-amended soils than those grown on unamended soils, the values were not significantly different (**Table 4**). The greatest increase was observed for the root nodules per plant (RNCCP) and shoot dry mass (SDM), 51.5% and 38.1 %, respectively. There was no significant block effect on the plant growth parameters.

Table 4. Plant growth parameters. Values are means of plant stem height (SH), branches per plant (BPP), leaves per plant (LPP), leaf area index (LAI), leaf chlorophyll content (LCC), root length per plant (RLPP), root nodules per plant (RNCPP), shoot dry mass (SDM), root dry mass (RDM), root to shoot ratio (RDM:SDM). The percentage change represents increase or decrease between Black-eyed peas (BEP) plants grown on soils with or without EPS amendments.

Treatment	SH (cm)	BPP	LPP	LAI	LCC (Spad units)	RLPP (cm)	RNCPP	SDM (g)	RDM (g)	RDM:SDM
EPS + BEP	44.0	3.9	56.9	4.32	61.3	14.4	25.3	26.8	1.40	0.05
BEP	43.1	3.7	55.9	4.13	59.9	13.7	16.7	19.4	1.37	0.07
% change	2.1	5.4	1.8	4.6	2.3	5.1	51.5	38.1	2.2	−28.6
p-value Block	.31	.054	.03	.14	.09	.29	.13	.96	.77	.47
p-value treatment	.69	.42	.54	.41	.26	.39	.022	.24	.91	.57

p-values more than 0.05 indicate no significant block or treatment effect on the plant growth parameters.

The leaf chlorophyll content was monitored for BEPs grown in soils with or without EPS. Results indicated that EPS might have increased the chlorophyll content of the leaves. For both plants grown on soils with or without EPS, the leaf chlorophyll content dropped 40 days after the start of seed germination (Figure 4).

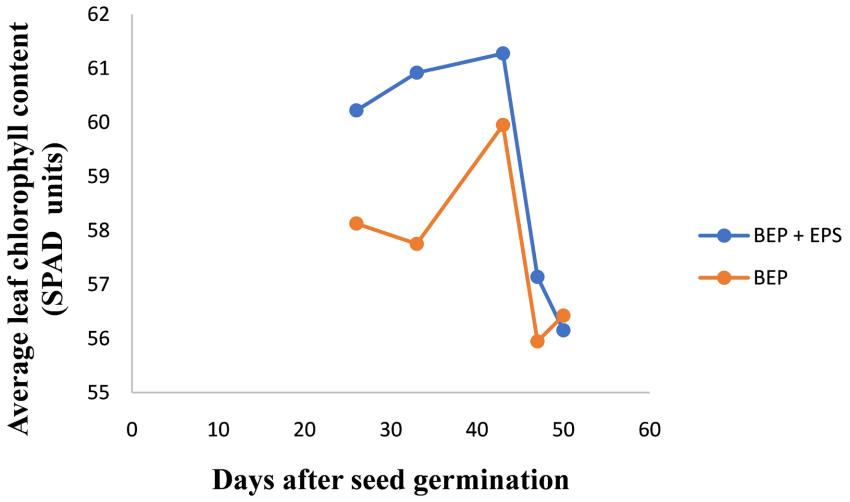


Figure 4. Variation of leaf chlorophyll content in Black-eyed pea plants (BEP) grown on EPS-amended (blue line) and un-amended (orange line) soils.

Our results also indicated that the average number of pods per plant, number of seeds per pod, and average pod weight were higher for BEPs grown on EPS-amended soil than on unamended soil, though not significantly different (Table 5). The highest percentage increase was on the average number of pods per plant, and the lowest was the average seed weight for plants grown on amended soils compared to plants on unamended soils. The seed yield of BEPs grown on soils amended with EPS increased by 22 % compared to the control.

Table 5. Pod and seed yield of Black-eyed peas (BEP) grown on soils with or without EPS.

Treatment	Average pods per plant	Average number of seeds per plant	Average pod weight (g)	Average seed weight (g)	Seed yield (kg/ha)
EPS + BEP	24.55 (±2.4)	7.72 (± 0.4)	2.24 (± 0.26)	0.225 (± 0.013)	3827
BEP	22.53 (± 2.9)	7.39 (± 0.3)	2.07 (± 0.06)	0.223 (± 0.007)	3137
% increase	8.96	4.46	8.21	.89	22.0
p-value Block	.908	.319	.618	.910	
p-value Treatment	.559	.274	.443	.906	

p-values > 0.05 indicate no significant block or treatment effect on the plant yield parameters.

4. Discussion

This was a field experiment to evaluate the effects of *R. tropici*-derived EPS on the

germination, growth, and yield of Black-eyed peas. In a previous greenhouse study, we had established that EPS delayed seed germination in potted soils but increased plant biomass, particularly root density. At 0.5% EPS (w/w) in soils, microbial biomass and root biomass were significantly reduced compared to the control, whereas 0.1% EPS increased shoot and root biomass [29]. In this experiment, the soil was amended at 10 cm depth with 0.1% (w/w) EPS and evaluated for its effect on soil properties and growth and yield of Black-eyed peas.

4.1. Effects of EPS on Soil Biological and Chemical Fertility Indices

4.1.1. Effect of EPS on Soil Chemical Properties

Although amending soils with EPS slightly increased soil pH from 6.4 to 6.8 after a week, it did not affect CEC, SOM, and pH after 49 days. This potentially indicates that certain soil chemical properties may not respond to organic amendments in the short term. However, there was an increase in POx-C (active carbon) and nitrate nitrogen, attributed to an increase in soil microbial activity and N mineralization potentials. The decrease in NO_3-N in EPS-amended soils grown with BEP (**Table 1b**) is considered due to plant uptake.

4.1.2. Effects of EPS and Black-Eyed Pea Growth on Soil CO_2 Flux

Soil is both a source and a sink of CO_2 exchange and aids in carbon sequestration. The role of cover crops and soil amendments in the terrestrial carbon cycle has been of interest for years [30]. Cover crops have been shown to have great potential in the transfer of atmospheric carbon dioxide to soil carbon [14]. Soil conservation and management practices that impact CO_2 release to the atmosphere have been implicated in global climate change [31]. This study shows that growing BEPs in EPS amended soils increased soil CO_2 flux. The impact of EPS and BEP growth on CO_2 flux was also affected by the measurement time indicating that other factor(s) do control soil CO_2 flux. When the soils were amended with *R. tropici* EPS and planted to BEP cover crop, soil CO_2 flux became less dependent on soil temperature and more dependent on soil moisture (**Table 2**). A weak positive correlation was observed between soil CO_2 flux and soil temperature, and a moderate correlation with soil moisture when the soil was amended with EPS in the absence of BEPs, suggesting that CO_2 flux in EPS-amended soils is less sensitive to changes in soil temperature and more sensitive to soil moisture. The average temperature of the EPS-amended soils was 35.4˚C, which is close to the 35 ˚C used in models [32] and far from the optimum of 38.5 to 46.0 ˚C known to affect soil respiration [33]. A previous study showed that a negative correlation (low CO_2 flux at higher soil temperatures) observed between soil CO_2 flux and soil temperature was attributed to lower soil moisture content [10]. While soil carbon dioxide emissions have been attributed to increased soil temperature [34], the soil temperatures recorded in this study (31.4˚C - 38.4˚C) were below the range of 38.5˚C to 46.0˚C known to affect CO_2 production [33]. Soil respiration is a combination of root and microbial respiration, where root respiration is estimated to contribute over 50%, depending on the season and vegetation type [35]. Based on this

study, EPS increased soil microbial biomass (total PLFA), indicating that the increase in soil flux was partly due to an increase in soil microbial activity. Root exudates are known to attract microorganisms. The CO_2 flux was generally higher for EPS-amended soils with BEP cover crops than for amended soils without BEP (except on day 48) (**Figure 1**). This can be attributed to a combined increase in root respiration and microbial activity compared to the control. The effects of EPS and BEP growth on CO_2 flux were significant only on certain days of measurements. The marginal correlation of CO_2 flux with soil temperature and the stronger correlations with soil moisture content (**Table 2**) indicate that soil moisture is a regulatory factor on the impacts of EPS soil amendments and cover crop growth on soil CO_2 flux. In this study, soil CO_2 flux was measured for 65 days. A longer monitoring period spanning several seasons may give a clearer picture of the effect of EPS on soil CO_2 flux and its potential impact on climate change.

4.1.3. Effects of EPS and Black-Eyed Pea Growth on Soil Microbial Biomass

Organic amendments usually increase soil microbial biomass, depending on other soil indices and management practices [36]. In this study, the emphasis was on the percentage increase in the microbial biomass compared to the control rather than the absolute PLFA values. Some studies have shown that organic amendments did not significantly change soil microbial biomass with season [9] [37]. However, our study showed that EPS increased the biomass of all microbial groups within 65 days of the study. The EPS-amended soils showed the lowest soil microbial fluctuations compared to un-amended soils or un-amended soils planted to BEP. The fact that EPS is important in the nutritional support of soil microbes [3] may encourage healthy microbial growth. The large percentage increase in GN bacteria and AMF at the early stage of the growth of the cover crops on EPS-amended soil (**Figure 2a**) may be critical in plant root development and plant nutrition. Proliferations of AMF in soils form symbiotic relationships with a host plant. In the AMF-plant symbiotic relationship, the fungal hyphae obtain sugars from the host plant's roots and, in turn, extend the root system of the plant, assisting the plant to absorb water and key nutrients [38]. Fungi (AMF) have been shown to increase soil aggregate stability via entanglements of their hyphae and the release of glomalin-related soil protein [39] [40]. The increase in soil aggregate stability by *R. tropici* EPS [12] may, in part, be due to the stimulating effects of EPS on the growth of AMF.

PLFA profiles reflect microbial adaptation to environmental stressors. When Gram-negative bacteria are under ecological stress, they change some of their monounsaturated fatty acids to cyclopropane fatty acids [27]. Our results showed that amending soils with EPS increased the ratio of monounsaturated fatty acids to cyclopropane fatty acids, indicating a reduction in microbial stress (Gram-negative stress). At the early stages of EPS application and BEP growth, EPS and BEP shifted the microbial population towards GN bacteria. At plant maturity, BEP favored the growth of Gram-negative (GN) bacteria and AMF, whereas EPS shifted the microbial population from GN to Gram-positive (GP) bacteria (**Table 3**). The

increase in the fungi to bacterial biomass ratio may contribute to an improvement in the general soil health and performance of BEPs. These shifts in microbial group populations at various stages of plant growth indicate the various roles the microorganisms play in soil and the plant's nutrition. This highlights the importance of monitoring the changes in soil microbial community composition during plant growth. Gram-negative bacteria play significant roles in the protein nutrition of plant legumes, while Gram-positive bacteria are linked to the mineralization of more recalcitrant organic materials partially decomposed by fungi or GN bacteria [26]. Some GN bacteria (Rhizobia), which grow inside the root nodules of legumes, can absorb and fix atmospheric nitrogen to plant usable ammonia. Our study does indicate that amending soils with EPS significantly increased the root nodule counts per plant (**Table 4**). Some studies have also implicated some strains of GN bacteria in the phosphorus nutrition of legume plants [41]. Nitrogen and phosphorus are key elements in proteins; thus, the shift of microbial community composition from GP to GN bacteria throughout the growth and maturation of BEPs may be important in the seed nutrient quality. The variation in soil moisture is likely to also affect the response of soil microbial biomass to EPS amendments and BEP growth. It is important to note that the experiment was conducted between June 30 and August 31 under free rain, without irrigation.

4.2. Effects of EPS on Seed Germination, Growth, and Yield of Black-Eyed Peas

It was observed that EPS increased seed germination rates, at 75.4% for amended soils and 71% for unamended soils. Relatively low seed germination was attributed mainly to a lack of moisture and damage to seedlings by rodents. The EPS solution provided the initial soil moisture for the amended soil, while an equivalent amount of tap water was used on the unamended plot. Thereafter, the plants were allowed to grow under field conditions without irrigation. In a previous greenhouse study, it was observed that EPS delayed seed germination in soil [29]. We attributed the delay in seed germination in amended potted soils to water movement to the surface by capillary action, carrying along EPS that dried and hardened at the soil surface and prevented the seeds from sprouting. However, it has been demonstrated that EPS improved the germination rate of green bean seeds [13]. In the study, the seeds were allowed to germinate in petri dishes lined with paper towels soaked in an EPS solution before being transferred to pots filled with soil. Under field conditions, any EPS transported to the soil surface likely spreads over a larger surface to a thickness that still allows the seedlings to sprout above the ground. Extracellular polymeric substances are known to conserve water [42], which might explain the greater seed germination rates observed in EPS-amended soil.

The plant growth parameters were estimated at the start of flowering when the plants were at the peak growth period with little or no leaf fall. The shoot biomass of amended plants increased by 38.1%, while the root biomass increased by 2.2 %

compared to unamended plants (**Table 4**). Also, the stem heights and root lengths of EPS-amended plants increased by 2.1% and 5.1%, respectively, compared to the unamended plants. The challenge of recovering the roots from the soil under field conditions may explain the relatively low root biomass values recorded. The root:shoot dry mass ratio was higher for unamended plants. This was attributed to a large increase in shoot biomass rather than a decrease in root biomass of plants grown on EPS-amended soil. The increase in shoot biomass of EPS-amended plants corresponded to an increase in leaf area index (LAI) and chlorophyll content compared to unamended plants (**Table 4** and **Figure 4**). Leaf area index and leaf chlorophyll content are indicative of the photosynthetic potential of a plant. An increase in LAI leads to a decrease in surface albedo and ground evaporation, which are critical variables in global climate change [43]. Chlorophyll represents an important pigment for photosynthesis, which is the main source of energy for plant growth. Although the leaf chlorophyll content was higher in plants grown on EPS-amended soils, the biopolymer did not seem to affect the onset of chlorosis since the leaf chlorophyll content dropped sharply for both amended and unamended plants forty (40) days after sprouting (**Figure 4**). While root biomass is an important factor in carbon sequestration [44], most soil carbon is derived from recent photosynthesis that takes carbon from the leaves to the root structures and to the soil via root exudates [45]. Because of its stimulating effects on root growth, it is reasonable to suggest that at a 0.1% (w/w) rate in soils, EPS will increase the carbon sequestration potential of BEPs. Also, the improvement of the canopy of the cover crop and the close association of plant roots with soil particles endear EPS in soil erosion control.

The most profound effect of EPS on the growth of BEPs was in root nodulation, with a 51.5% increase compared to plants on unamended soils. This is to suggest that *R. tropici* EPS stimulates the formation of root nodules to provide the habitat for the symbiotic nitrogen-fixing rhizobia bacteria in BEPs, which is important in the protein nutrition of the plant. Leguminous roots secrete flavonoids, which attract rhizobia towards the root [46]. Rhizobia are housed within the nodules and use glucose provided by the legume to convert atmospheric nitrogen to ammonia, used by the plant for the synthesis of proteins and other N-containing organic molecules [47]. The introduction of exogenous EPS to soils likely attracts Gram-negative bacteria and protects them from adverse soil conditions, while the bacteria will, in turn, induce the nodulation of the plant roots.

The effects of EPS on seed yield were assessed by considering the average number of pods per plant, the number of seeds per pod, and average seed weight. The number of pods per plant increased by 8.9%, probably due to an increase in the number of branches (5.4%) for plants grown on amended soils compared to the unamended soils. Although the average seed weight was almost the same for treated and untreated plants, the grain yield was higher for treated plants because of a higher number of pods per plant and seeds per pod. It is important to note that BEPs are used as food at different stages of their growth, with green leaves

before flowering, green pods, and dry seeds at maturity. The increase in shoot biomass would imply an increase in vegetative yield. Although the seed yield of BEPs grown on soils amended with EPS increased by 22% compared to the control (Table 5), further studies are needed to investigate the effect of long-term application of EPS on the yield of BEPs or its residual effects on the yield of some other crop in a crop rotation system. Such a study will determine a cost-benefit analysis of using microbial EPS in agricultural systems

5. Conclusion

Microbial extracellular polymeric substances (EPS) have been employed in agriculture to improve soil fertility indices and erosion control. Limited studies have been conducted on their effects on soil biological and biochemical attributes, plant growth, and crop yield. Although amending soils with *R. tropici*-derived EPS in this study did not affect most soils' chemical attributes in the short term, it increased soil flux, active carbon, total nitrogen, nitrate-N, microbial biomass, and the growth, root nodulation, and yield of Black-eyed peas. At the early stage of applied EPS in soil, the microbial population shifted from GP to GN bacteria, while at later stages, the population shifted from GN to GP bacteria. The growth of the BEP cover crop shifted the microbial population from GP to GN bacteria and favored the growth of AMF. The dynamic patterns of soil CO_2 flux and microbial responses to EPS amendments and BEP growth indicate the complexity of predicting soil processes. Thus, further research is vital to assess the relative contributions of crops and soil amendments to soil biological fertility indices. This study provides insights into the short-term response patterns of soil properties and the growth of a cover crop after amendments with microbial EPS.

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Conflicts of Interest

The authors declare that they have no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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