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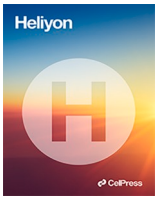

Research article

Inoculation of *Erythrina brucei* with plant-beneficial microbial consortia enhanced its growth and improved soil nitrogen and phosphorous status when applied as green manure

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ABSTRACT

Erythrina brucei has been applied as a green manure to improve soil fertility in southern Ethiopia. It has been nodulated by indigenous rhizobia. The objectives of this study were to evaluate the effects of *E. brucei* inoculation with microbial consortia consisted of *Bradyrhizobium shewense*, *Acinetobacter soli* and arbuscular mycorrhizal fungi (AMF)on *E. brucei* growth, soil nitrogen and phosphorous status after application as a green manure.A field experiment was conducted by inoculating *E. Brucei* with different microbial consortia. *E. brucei* inoculated with the microbial consortia were grown for 150 days. Its shoot length was measured at 60, 90, 120 and 150 days after planting. Then, plants were uprooted and mulched as a green manure. The soil nitrogen, available phosphorous and soil organic matter analysis were done. The experimental design was completely randomized block design with eight treatments comprised of three replications. Inoculated treatments did not show a significant ($p < 0.05$) difference in shoot length in the first 60 days. However, shoot length was increased between 19.1 and 41.3 %, 10.5–43.4 % and 8.7–37.6 %, respectively at 90, 120 and 150 days. The soil organic matter was improved in both inoculated and un-inoculated treatments. The improvements in the soil organic matter of un-inoculated treatments may be due to the decomposition of un-inoculated plants biomass in the soil. The *B. shewense* inoculation improved the soil nitrogen by 17 %. The soil phosphorous was improved in 57 % of inoculated treatments. The inoculation of *E. brucei* with microbial consortia enhanced its growth and improved soil fertility when applied as a green manure. Inoculating the green manure legumes with symbiotically effective rhizobia and plant-beneficial microbes can enhance the growth of *E. brucei* and its nutrient uptake.

1. Introduction

Erythrina brucei is a woody legume and multi-purpose tree commonly used by farmers for the soil fertility improvement [1]. The plant is commonly found in different habitats and widely distributed in the southern and south western parts of Ethiopia. The smallholder farmers in aforementioned parts of Ethiopia widely use the biomass of *E. brucei* mainly as an organic nutrient source. These farmers integrate their farming practices with this plant by using as a shade tree for coffee plantation and as inputs in soil fertility and

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crop yield improvement [2]. It is because the plant has peculiar agro-forestry characteristics such as rapid establishment, high rate of litter production, fast rate of litter decomposition and mineralization, profuse re-growth after cutting and coppicing and, rapid recovery after a period of sustained drought [3,4].

E. brucei forms a symbiotic nitrogen fixing association with the soil bacteria known as rhizobia [5–8].Its symbiotic nitrogen fixing associations with diverse groups of soil bacteria such as *Bradyrhizobium* [6,7,7,8], *Rhizobium* [6,7,9] and *Mesorhizobium* [5,6] has been well documented. The other non-rhizobial endophytic soil bacterial species like, *Rahnella aquatilis*, *Enterobacter* [5,6],*Agrobacterium* [5, 6,9], *Staphylococcus edaphicus*, *Staphylococcus cohnii* sub sp. *urealyticus*, *Bacillus luti*, *Paenibacillus peoriae*, *Enterobacter ludwigii* and *Stenotrophomonas maltophilia* [8], *Acinetobacter soli*, *Achromobacter xylosoxidans*, *Bacillus thuringiensis* and *Gluconobacter cerinus* [10], were also recovered from root nodules of this plant. As the most leguminous plants do, *E. brucei* forms dual symbiotic associations with both rhizobia and arbuscular mycorrhizal fungi (AMF). Its symbiotic association with AMF has been reported by several scholars [11–13]. Berza et al. [13] have reported several AMF genera and species associated with this plant. These genera include *Glomus*, *Pacispora, Acaulospora*, *Septoglomus*, *Racocetra*, *Dentiscutata*, *Archaeospora*, *Ambispora*, *Scutellospora*, *Diversispora* and *Cetraspora*.

The availability of soil nitrogen (N) and phosphorous (P) can be enhanced in the rhizosphere of leguminous plants through nitrogen fixation and mobilization of organic and inorganic phosphates.The mobilized nutrients like N, P and other macro and micro nutrients are translocated by the hyphae of AMF. The synergetic interactions among rhizobia, rhizobacteria and AM fungi have enhanced the soil nutrient management in the legumes rhizosphere [14,15]. The AM fungi directly take up inorganic P and nitrogen from the soil beyond the depletion zone and transport to the host plants [16]. Enhanced legume plant growth, biomass production and nitrogen uptake has been reported due to improved legume-rhizobium-AMF symbiosis. Similarly, dual inoculation of *E. brucei* with *Bradyrhizobium* species and AMF species exhibited 16.9 %–45.3 % improvement in shoot dry weight and 41.6 %–75 % increment in shoot total nitrogen under greenhouse conditions [11]. In addition, the inoculation of microbial consortia consisted of *Bradyrhizobium shewense*, *Acinetobacter soli*, *Glomus* sp.1 and *Acaulospora* sp.1 has increased *E. brucei* shoots length and shoots dry weight by 140 % and 260 %, respectively compared to un-inoculated control plants under greenhouse conditions [17]. Furthermore, a dual inoculation consisted of *Bradyrhizobium shewense* and *Acinetobacter soli* has increased the *E. brucei* shoot nitrogen content by 260 % and anotherdual inoculation composed *Bradyrhizobium shewense* and *Glomus*sp.1 has similarly increased the *E. brucei* shoot phosphorous content by 1200 %, compared to un-inoculated plants [17].

With regard to biomass production, *Erythrina* species produce up to 50 kg fodder per tree per year [18] and the annual litter production of *E. brucei* tree is about 929 g per unit area of crown of a tree [19]. The fast decomposition and mineralization rate of *E. brucei* litter is very crucial to release organic nutrient sources for accompanying crops in the case of intercropping and/or those plants grown on the soils containing mulched and decomposed plant material. According to Haile et al. [18] and Abay [20], the decomposition of *E. brucei* biomass released nitrogen in the form of NH_4^+-N and NO_3^- –N. The fast litter decomposition and mineralization of *E, brucei* biomass could directly be related to its high total nitrogen (TN) content, low lignin, low cellulose and polyphenol content [20]. The organic nutrient sources containing high N, low lignin and polyphenol contents release N and other nutrients faster when compared to organic plant materials containing higher lignin and polyphenol contents [21].In this context, based on the N, lignin and polyphenol contents, *E. brucei* is considered a high quality organic plant nutrient material for agro-forestry use. Haile et *al.* [18] have recorded wheat grain and straw yield increment by 127 % and 194 %, respectively due to the application of 2.5 tons of *E. brucei* dry matter per hectare.

The smallholder farmers in the southern Ethiopia, who do not have the capacity to purchase and applying chemical fertilizers, have been planting *E. brucei* as an alternative organic nutrient source. They commonly grow *E. brucei* plants and leave in their farms, home gardens and also use as live fences around their land boundaries. During cereal crops cultivation seasons, these farmers cut or prune *E. brucei* plants and harvest their biomass and use as a mulching material or green manure during land preparations. These smallholder farmers have been using the plant biomass spontaneous enriched due to nitrogen fixation involving indigenous rhizobia. The same holds true for the phosphate solubilizing rhizobacteria and the AMF, when phosphorous enrichment in the plant biomass is concerned. However, it possible to improve the nitrogen and phosphorous content of *E. brucei* biomass by 1) inoculating with the symbiotically effective rhizobium species; 2) dually inoculating the symbiotically effective rhizobium species and the AMF; 3) dually inoculating the symbiotically effective rhizobium species and phosphate solubilizing rhizobacteria and 4) inoculating the consortia of symbiotically effective rhizobium species, rhizobacteria with multiple plant growth promoting traits and AMF under field conditions. Hence, there is dearth of information with regards to the microbial consortia application to enhance the *E. brucei* growth and development so as to improve its symbiotic association with rhizobium and AM fungi to improve N and P content in its biomass under field conditions. Therefore, the objectives of this study were to evaluate the effects of the inoculation of *E. brucei* with the microbial consortia consisted of symbiotically effective *Bradyrhizobium* species, rhizobacteria with multiple plant growth promoting traits and AMF species on plant growth and nutrients uptake status when applied as green manure in the field condition.

2. Materials and methods

2.1. Description of the study site and determination of existence of indigenous E. brucei nodulating bacteria

The field experiment was conducted at Gallika Flowers Farm P.L.C, Menagesha, Ethiopia. The experimental site is situated at 09° 031' 43.315" N and 38° 33' 93.111" E with an altitude of 2576 m.a.s.l. The mean annual temperature was between 18 °C to 22 °C and mean annual precipitation was between 900 mm and 1562 mm. The experiment was conducted between August 2017 and February 2018. In the experimental field, hydrangea flower was cultivated and harvested before this experiment. The presence and/or absence of native *Erythrina brucei* plant nodulating rhizobia in the experimental field was determined by planting surface sterilized and

Table 1
Microorganisms, their scientific names, Accession numbers and phytobeneficial properties.

Microbes	Scientific name	Accession number	Phytobeneficial properties								References
			N fixation (SE%)	IAA production (μg/mL)	P solubilization (mg/L)	HCN	HN_3	Chitinase	protease	Lipase	
AU27	*Bradyrhizobium shewense*	MK370570	126	–	1 (SI)	–	–	–	–	–	Berza et al., 2021a
AU4	*Acinetobacter soli*	MK370560	–	171.65	120.36	+	+	–	+	+	Berza et al., 2022b
RG6	*Acinetobacter soli*	MK370561	–	147.28	112.82	+	+	+	+	+	Berza et al., 2022b
AMF1	*Glomus* sp.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	Berza et al., 2021b
AMF2	*Acaulospora* sp.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	Berza et al., 2021b

+ = Presence of a trait; - = absence of a trait; Relative symbiotic effectiveness (SE %); Phosphate solubilization index (SI); the phosphate source was $Ca_3(PO4)_2$; NA = not applicable.

germinated seeds of *E. brucei* on 1 % water agar. Eighteen (18) seedlings were planted at 10 m distance from each other starting from the field center in all directions. The plants were watered as required for three months and uprooted and checked for root nodulation. The physicochemical properties of the experimental field soil were determined.

2.2. Determination of soil physicochemical properties

The field soil pH was determined according to the methods described in Ziadin and Tran [22].The determination of soil organic matter was carried out according to the methods described in Walkley and Black [23], whereas the total nitrogen (TN) determination was conducted following the Kjeldahl method [24].The Olsen method was employed to determine available phosphorous [25].

2.3. Source of microorganisms and the target plant seeds

The bacterial species AU27 (*Bradyrhizobium shewense*) and the strains AU4 and RG6 (*Acinetobacter soli*) were obtained from the culture collections in the Applied Microbiology laboratory, Department of Microbial, Cellular and Molecular Biology, Addis Ababa University (Table 1). These bacteria species were previously isolated from the root nodules of *E. brucei* [8,10]. They were previously identified to the species level using 16 S rRNA gene sequence analysis and the partial 16 S rRNA gene sequences of these bacteria species were deposited in NCBI database under accession numbers MK370560 for AU4, MK370570 for AU27 and MK370561 for RG6 [8,10]. The symbiotic effectiveness of AU27 (*Bradyrhizobium shewense*) was previously confirmed [17]. The plant-beneficial traits of the bacterial species are presented in Table 1. Similarly, the arbuscular mycorrhizal fungal spores AMF1 (*Glomus* sp.1) and AMF2 (*Acaulospora* sp.1)(Table 1) were also previously obtained from the rhizosphere of *E. brucei* [13]. The *E. brucei* seeds were obtained under *E.brucei* plants at Addis Ababa University, College of Natural and Computational sciences. The seeds were sorted and air dried. The uniform and healthy seeds were surface sterilized and germinated on 1 % water agar (w/v) for seven days and the seedlings were used in the field experiment.

2.4. The inoculant preparation

The compatibility between AU27 and AU4 was previously studied [17], whereas the compatibility studies between AU27 and RG6 and AU4 and RG6 were conducted following cross streaking method as described in Santiago et al. [26]. AU27 was grown to log phase in yeast extract mannitol (YEM) broth comprised of (g/L) [yeast, 0.5; D-mannitol, 10; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1] for 72 h at 28 °C. Similarly, the root nodule endophytes AU4 and RG6 were also grown to exponential phase in nutrient broth at 28 °C for 72 h. Arbuscular mycorrhizal fungi inoculum was multiplied according to the methods described in Berza et al. [17]. In brief, both *Glomus*sp.1 and *Acaulospora* sp.1 spores were multiplied using maize (*Zea mays*) host plant in sterile soil-sand mixture (2:1) under greenhouse condition for 90 days. After 90 days, AMF spore density per 100 g dry soil was quantified using sucrose density gradient centrifugation technique according to the methods described in Brundrett et al. [27] and the AMF root length colonization was also determined for maize roots according to the methods described in McGonigle et al. [28].Then the crude inocula comprised of *Glomus* sp.1 and *Acaulospora*sp.1 spores and colonized maize root segments were used. The crude inocula consisted of 120 *Glomus* sp.1 spores per 100 g soil-sand mixture and 68 % *Glomus* sp.1 colonized maize roots segments, and 85 *Acaulospora* sp.1 spores per 100 g soil-sand mixture and 52 % *Acaulospora* sp.1 colonized maize root segments [17].

2.5. The experimental design

The experimental design was completely randomized block design (CRBD). The treatments were consisted of:
T1- AU27;
T2- AU27 + AU4;
T3- AU27 + RG6;
T4- AU27 + AMF1;
T5- AU27 + AMF2;
T6- AU27 + AU4 + AMF1;
T7- AU27 + RG6 + AMF2 and.
T8-un-inoculated control.
Each treatment consisted of three replications. Each plot size was 3 m × 1 m and equidistant plant spacing method was used. The experiment consisted of 3 blocks, 24 plots.

2.6. Preparation of plots, transplanting seedlings and inoculation of microorganisms

Twelve small holes having 10 cm depth and 5 cm diameter were dug in each plot. Into the treatments that consisted of AMF inoculation (T4 and T5), 150g of crude inocula of *Glomus* sp.1 or *Acaulospora* sp.1 was individually placed into prepared holes and each 75 g crude inocula of *Glomus* sp.1 and *Acaulospora* sp.1 (150 g) were placed into dual inoculation treatments consisted of AMF1 and AMF2 (T6 and T7).The *E. brucei* seedlings were transplanted into each holes including un-inoculated treatment (T8). There were 12 plants per plot and a total of 288 *E. brucei* plants were included in the experiment. Following successful establishment, each seedling except (T8) was inoculated with the bacteria species as follows; 2 ml YEM broth culture of AU27 (10^8 CFU)was inoculated into

treatments involving AU27 and AU27 + AMF (T1, T4 and T5). Similarly, 1 ml nutrient broth culture of each of phosphate solubilizing and IAA producing bacteria (Table 1) (AU4 & RG6) were inoculated in to the treatments involving AU4 and RG6 (T2,T3,T6 and T7). The plants were watered as required twice per week during dry seasons, once a week during semi dry and no watering at all during wet seasons. Plant height was recorded for randomly selected six plants per treatment at 90, 120 and 150 days after planting (DAP). All the plants were uprooted at 150 DAP in their respective plots and nodulation was checked.

2.7. Plant uprooting, mulching and determination of the soil nutrient status

The uprooted plants were mulched and distributed uniformly in each plot and left to decompose. The fresh weight of plant biomass in each plot was between 4 and 5 kg. The plant biomass was completely decomposed between 40 and 60 days after uprooting and mulching. After complete decomposition, each plot was carefully ploughed to mix the decomposed plant biomass with the top soil. Then after a month, soil sample was collected (0–10 cm depth) from four points in each plot and pooled into single composite sample per plot and finally pooled per treatment. The soil nutrient analysis was outsourced to CROPNUTS Laboratory services, Nairobi, Kenya.

2.7.1. Data analysis

One-way ANOVA was employed to test the significant differences among different parameters within and between inoculation treatments using SAS version 9.4. The Tukey's HSD multiple range test was conducted to test for mean separation ($p < 0.05$).

3. Results

3.1. Exploration of indigenous E. brucei nodulating bacteria and compatibility study among bacterial species

The exploration studies conducted by planting *E. brucei* plant to check the existence of native *E. brucei* nodulating bacteria in the experimental field revealed the absence of such bacteria in the experimental field. This was confirmed by the fact that none of the *E. brucei* plants produced nodules on their roots (Supplementary Fig. 1).All the 18 planted *E. brucei* seedlings did not show nodulation. Therefore, the presence of nodules on the roots of *E. brucei* in the later field experiments after inoculation of our nodulating bacteria (AU27)was attributed to the inoculation with AU27 (Supplementary Fig. 5). In addition, the compatibility study conducted among bacteria species through cross streaking experiment exhibited that all the bacterial species included in this experiment were compatible (Supplementary Fig. 2), as the bacteria were able to grow even on the crossing points. These bacteria were compatible and could grow synergistically in the soil.

3.2. Soil nutrient status before the experiment and microorganism

The soil nutrient analysis revealed that before planting *E. brucei* plant, the field soil pH was 6.7 (Table 2) which shows that the field was slightly acidic. The available phosphorous and the total nitrogen of the field soil were 191 (mg/kg) and 0.19 % (Table 2), respectively. In addition, the field soil organic matter was about 4.46 % (Table 2).Moreover, this bacterium had inorganic phosphate solubilization capability with phosphate solubilization index (1.0) (Table 1).

3.3. Plant growth measurement

All the transplanted seedlings were successfully established in the field conditions (Table 3). In this experiment, we did not record a significant ($p > 0.05$) difference in plant height/shoot length among inoculation treatments in the first 60 days after planting (DAP). In the three months of growth, the inoculated and un-inoculated plants began to exhibit differences in their shoot length (Fig. 1). We recorded significant ($p < 0.05$) differences in plant shoot length among inoculated treatments at 90 DAP. The highest shoot length was recorded due to the inoculation of AU27 + AMF1 (53 cm) followed by 51.7 cm which was as a result of the inoculation with AU27 + AMF2 (Fig. 1). Similarly, a significant improvement in shoot length was recorded due to the microbial consortia inoculation. The inoculation of the microbial consortia consisted of AU27 + RG6 + AMF2 exhibited 49.7 cm shoot length followed by 48.6 cm shoot

Table 2
The field soil nutrient status before application of green manure.

The major nutrients of the soil used in the field experiment	
Parameters(units)	Values
pH **(1:2.5)**	6.7
EC (us/cm)	97.8
Avail.P (mg/kg)	191
K+ (mg/kg)	912
Ca++ (mg/kg)	3270
Mg++ (mg/kg)	718
CEC (meq/100)	27.1
TN (%)	0.19
OM (%)	4.46

Table 3
The status of soil pH, available phosphorous and organic carbon after application and decomposition of mulched green manure enriched by inoculation of consortia of microorganisms.

Treatments	pH(H_2O)	P(PPM)	OC (%)
AU27	6.01cd	169ed	3.77b
AU27+ AU4	6.21bcd	298a	3.66cd
AU27+RG6	6.41ba	233b	3.64d
AU27+AMF1	6.12cd	202c	3.81a
AU27+AMF2	6.24BCE	169ed	3.69 cb
AU27+AU4+AMF1	5.94d	207c	3.73c
AU27+RG6+AMF2	6.05cd	158ef	3.63d
Non Inoculated	6.08cd	151f	3.64cd
Unplanted Soil	6.58a	180d	3.42e

Mean values with the same letter in the in the same column do not differ among themselves in the ANOVA Duncan test ($p < 0.01$).

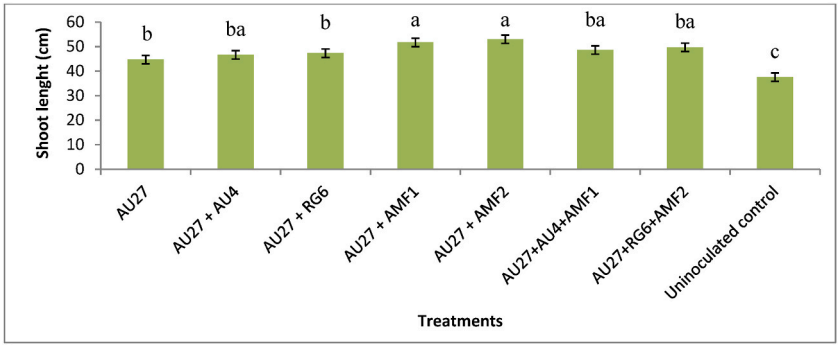

Fig. 1. Mean *Erythrina brucei* shoot length (cm) 90 days after planting in the field inoculated with microbial consortia. Different letters above bars indicate significant difference at ($p < 0.01$) according to LSD multiple range test.

length which was due to the consortium of AU27 + AU4 + AMF1. The un-inoculated control plants exhibited the least shoot length (37.5 cm) (Fig. 1).

At the 90 DAP, between 19.2 % and 41.3 % increment in shoot length was recorded compared to the un-inoculated control plants (Fig. 1). The highest shoot length increments (41.3 %) and (37.8 %) were recorded due to inoculation withAU27 + AMF2 and AU27 + AMF1, respectively compared to the un-inoculated control treatments. The inoculation of the microbial consortia comprised of AU27 +RG6 +AMF2 and AU27 +AU4 +AMF1 and exhibited 32.5 % and 29.6 % shoot length increment, respectively compared to the un-inoculated control treatments.

The plant growth/shoot length continued to show a significant ($p < 0.05$) difference among treatments at 120 DAP. During this period, the highest shoot length was recorded in the inoculation treatments that involved AU27 + AU4 +AMF1 (73.3 cm) followed by AU27 + AMF2 (73.2 cm)(Fig. 2). In addition, treatments consisted of AU27 + AMF1 and AU27 + RG6 + AMF2 showed shoot length of 73 cm and 69.4 cm, respectively at 120 DAP. The plant shoot length increment between 10.5 % and 43.4 % was recorded compared to un-inoculated control plants during 120 DAP. The highest plant shoot length increment was recorded in the treatments of AU27 +

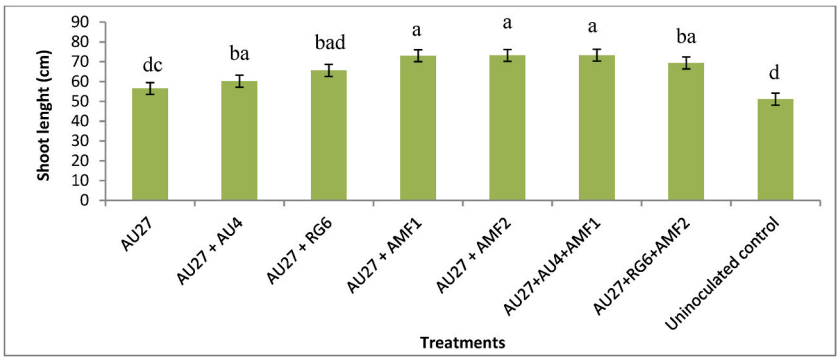

Fig. 2. Mean *Erythrina brucei* shoot length (cm) 120 days after planting in the field inoculated with microbial consortia. Different letters above bars indicate significant difference at ($p < 0.01$) according to LSD multiple range test.

AMF2 (43.4 %), AU27 +AU4 + AMF1 (43.3 %) followed by AU27 + AMF1 (42.8 %) compared to un-inoculated control treatments. In the present study, the highest shoot length increments were recorded in treatments consisted of AMF and PGPR dual inoculation or AMF and PGPR consortia inoculations compared to the PGPR or PGPR dual inoculations (Fig. 2).

At the 150 DAP, we recorded a significant ($p < 0.05$) difference in plant shoot length among treatments (Fig. 3). The highest plant shoot length (98 cm) was recorded in the treatments that consisted of AU27 + AMF1 followed by 94.1 cm which was due to AU27 + AMF2 inoculation (Fig. 3). The other inoculation treatments comprised of the microbial consortia of AMF and PGPR exhibited the next highest shoot length. The inoculation treatments consisted of AU27 + AU4 +AMF1 showed the shoot length of 93.1 cm; whereas AU27 + RG6 + AMF2 consortia inoculation showed 90.9 cm shoot length at 150 DAP. The inoculated treatments exhibited between 8.7 % and 37.6 % improvement in shoot length compared to un-inoculated treatments. The highest improvement in plant shoot length (37.6 %) was due to the inoculation of AU27 + AMF1, followed by 30.7 % due to AU27 + AU4 + AMF1 inoculation compared to un-inoculated control. The inoculation of this particular plant with AU27 + AMF2 improved shoot length by 27.9 %, whereas 27.6 % improvement in shoot length was recorded by AU27 + RG6 +AMF2 inoculation compared to un-inoculated treatment.

3.4. Soil nutrient status after mulching and decomposition of E. brucei biomass enriched with N and P using microbial consortia

All the treatments even the un-inoculated treatment, reduced the soil pH between 0.17 and 0.64 pH units compared to un-planted soils (Table 3). The un-inoculated plant treatments reduced the soil pH more than most of the inoculated treatments (Table 3). The highest pH reduction was recorded in the soils inoculated with consortia of AU27 + AU4 + AMF1 (0.64) pH units followed by 0.57 pH units due to AU27 inoculation (Table 3).

The highest available phosphorous (298 ppm) was recorded in AU27 + AU4 dual inoculated treatment followed by 233 ppm, which was due to AU27 + RG6 dual inoculation. All the inoculated treatments improved soil available phosphorous compared to un-inoculated treatments (Table 3). However, in this treatment, we observed two important points with available phosphorous compared to un-planted treatments. The un-inoculated treatments exhibited significantly ($p < 0.05$) lower available phosphorous compared to un-planted treatments (Table 3). About 57 % of the inoculated treatments showed significant ($p < 0.05$) improvements in the soil available phosphorous level (Table 3).The dual inoculations of *E. brucei* with AU27 + AU4 and AU27 + RG6 improved the soil available phosphorous by 97.4 % and 54.3 %, respectively compared to the un-inoculated control plants. Similarly, inoculations with AU27 + AU4 + *Glomus* sp.1 and AU27 + *Glomus* sp.1 increased the available phosphorous status by 37.1 % and 33.8 %, respectively compared to the un-inoculated treatments.

In this study, we also observed significant ($p < 0.05$) differences among treatments with regards to total nitrogen content of the soils after the decomposition of inoculated and enriched plants biomass. The highest soil total nitrogen content (0.24 %) was recorded in a single inoculation treatment that consisted of AU27 followed by the value of 0.21 % total nitrogen, which was due to the dual and consortium inoculation of AU27 + AU4, AU27 + AU4 + AMF1 and AU27 + RG6 + AMF2 (Fig. 4). The soil total nitrogen content was improved by 17 % in the AU27 inoculated plants compared to the un-inoculated treatments, whereas 2.4 % improvement in soil total nitrogen content was recorded due to the inoculation of AU27 + AU4, AU27 + AU4 + AMF1 and AU27 + RG6 + AMF2 compared to the un-inoculated controls.

The highest organic matter content (3.81 %) was recorded in the treatments that consisted of AU27 + AMF1 dual inoculation followed by 3.77 %,which was due to the single inoculation of AU27 compared to un-inoculated control. The smallest soil organic matter was recorded in the un-planted treatments. The *E. brucei* plants dually inoculated with AU27 + AMF1 improved the soil organic matter content by 11.4 % followed by 10.2 %, which was due to inoculation of AU27 compared to un-inoculated control (Fig. 5). We also observed significant ($p < 0.05$) differences among treatments in soil organic carbon contents. The highest soil organic carbon content (6.5 %) was recorded in the AU27 + AMF1 dual inoculation followed by 6.5 %, which is due to single inoculation of AU27 (Fig. 5). The smallest soil organic carbon (5.89 %) was recorded in the microbial consortium inoculation that consisted of AU27 + RG6

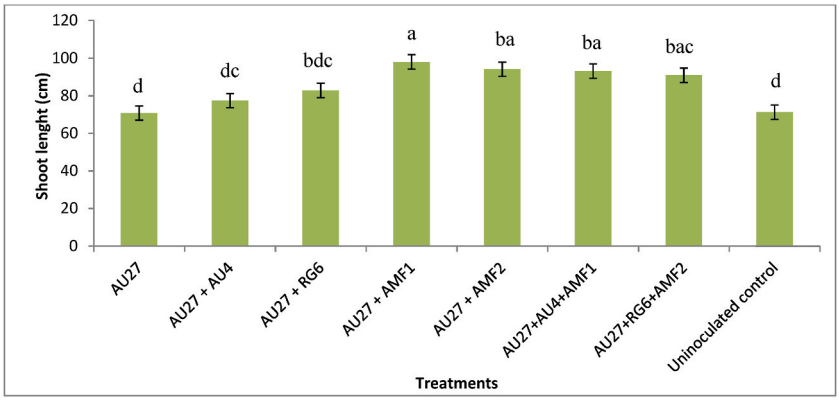

Fig. 3. Mean *Erythrina brucei* shoot length (cm) 150 days after planting in the field inoculated with microbial consortia. Different letters above bars indicate significant difference at ($p < 0.01$) according to LSD multiple range test.

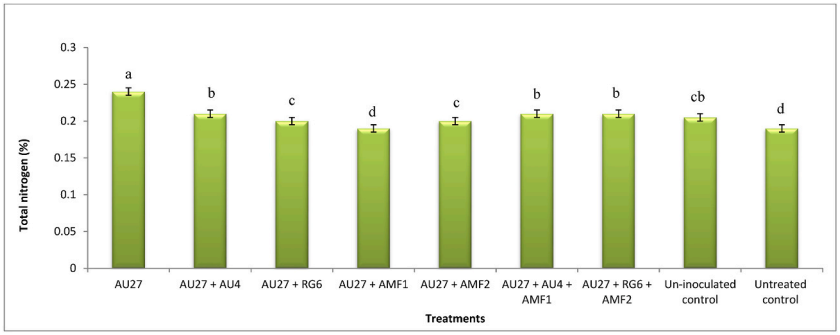

Fig. 4. Soil total nitrogen content (%) after microbial consortia inoculated *E. brucei* biomass mulches applied as green manure. Different letters above bars indicate significant difference at ($p < 0.01$) according to LSD multiple range test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

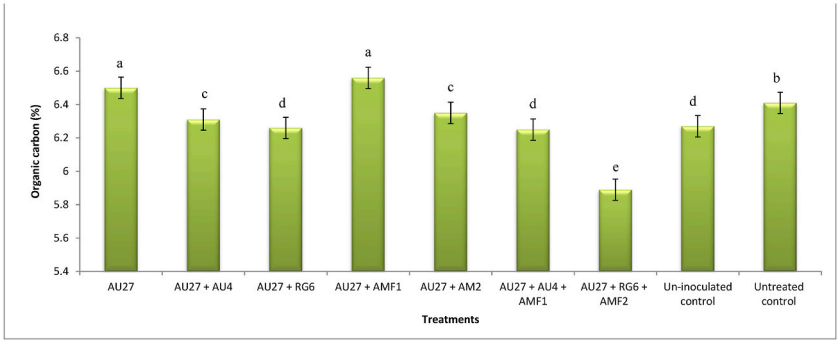

Fig. 5. Soil organic matter content (%) after microbial consortia inoculated *E. brucei* biomass mulches applied as green manure. Different letters above bars indicate significant difference at ($p < 0.01$) according to LSD multiple range test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

+ AMF2. The inoculation of AU27 + AMF1 improved the soil organic carbon content by 4.6 % followed by 3.6 %, which is due to single inoculation with AU27. The other treatments that consisted of AU27 + AU4 + AMF1 and AU27 + AMF2 improved the soil organic carbon content by 2.4 % and 1.3 %, respectively compared to the un-inoculated controls (Fig. 5).

4. Discussion

Like several other cover plant and green manure legumes, *E. brucei* has been used as a green manure woody legume in southern and southwestern Ethiopia. The *E. brucei* green manure/biomass has been applied as a part of low cost and affordable organic and sustainable agricultural input by smallholder farmers. This woody legume has been selected as a green manure and cover crop, because it is easily decomposable, its application improves soil fertility and crop yield [4]. The soil fertility improvement exhibited by *E. brucei* is associated with the dual symbiotic association of soil bacteria (rhizobia) and arbuscular mycorrhizal fungi. This dual symbiosis supports the legume plant growth by enhancing symbiotic nitrogen fixation which is very crucial for plant growth and development [8] and AMF help the legume plants by absorbing and translocating phosphorous, nitrogen, Zn, Fe and other micro and macro nutrients beyond root depletion zone [10]. Moreover, the nodules of this plant were occupied by endophytic bacteria endowed with multiple plant growth promoting traits such as IAA production and phosphate solubilization to assist symbiotic nitrogen fixation [5,6,10]. However, in the present context, the legumes applied as green manure are spontaneously nodulated with indigenous/native rhizobia, if they are present in the soil, whose symbiotic effectiveness and other plant-beneficial traits are not evaluated and confirmed. In this study, therefore, we investigated the effects of inoculating *E. brucei* with symbiotically effective rhizobium (*B. shewense*), multiple plant growth promoting rhizobacteria (*A. soli*) and AMF (*Glomus* sp.1 and *Acaulospora* sp.1) on plant growth and soil nutrient status after biomass application as a green manure.

Before the field experimentation, we had set up a small experiment to determine the presence of compatible native *E. brucei* nodulating bacteria in the field soil. However, we did not detect root nodules in the small field experiment planted with *E. brucei*. Several conditions can be mentioned for the absence of root nodules in legumes in field soils. The symbiotic partnership between legumes and nitrogen fixing bacteria is affected by several abiotic and biotic stressors. The well-established factors include presence of excessive nitrogen in the soil, soil acidity, soil high temperature, drought and the absence of compatible indigenous soil bacteria in the field [29]. In our case, all the abiotic conditions mentioned above are optimal for legume nodulation and the most probable reason

could be the absence of indigenous compatible soil bacteria in the experimental field soils. Finally, during the experimental stage, we inoculated the field soil with symbiotically effective rhizobia and recorded sufficient number of nodules.

In the present study, we evaluated plant growth in the field by measuring the shoot length of the inoculated plants and un-inoculated control plants. The microbial inputs did not exhibit a significant difference among treatments and even between inoculated and un-inoculated treatments in the first 30 and 60 days after planting (data not shown).The slow growth of the transplanted *E. brucei* seedlings in the field conditions could be attributed to different challenges in tolerating abiotic and biotic stressors in the field conditions. To mention some, the new root growth in recently transplanted seedlings has long been recognized as important factor in enhancing establishment and growth of seedlings. Therefore, sufficient root number, size and length are required before starting plant fast growth [30]. The root development related traits in turn determine efficient root colonization by AMF and nodulation by rhizobia. Accessing the plant nutrients and water from the soil in the field conditions requires well established root systems and hence the nutrient and available water status greatly affects plant performance in the field. However, Berza et al. [17] have recorded 39 % and 23 % increments in *E. brucei* shoot length due to inoculation of the microbial consortia comprised of AU27 +AU4 + *Glomus* sp.1 and AU27 +AU4 + *Glomus*sp.1 + *Acaulospora* sp.1, respectively compared to the un-inoculated control in the 30 days after planting in the greenhouse conditions. The same inoculation increased *E. brucei* shoot length by 42 % and 27.6 %, respectively compared to the un-inoculated control plants in the first 60 days after planting. In the field conditions, the more stressful growing conditions (for instance, competition, drought, herbivores) may minimize inoculation effects [31,32]. In this field experiments, we attempted to decrease the competitive effects of weeds by hand weeding, it is likely that the competitive pressure still exists and was greater in the field compared to the greenhouse conditions due to the larger seed bank in the unsterilized field soils [33]. In addition, the greater aboveground herbivores in the field were likely to decrease inoculation effects directly by removing aboveground biomass and potentially indirectly by inducing increased belowground growth [34]. Furthermore, drought in the field condition may also decrease the inoculation effects by decreasing plant growth, microbial growth and nutrient recycling rates [35].

The inoculated microbial inputs exhibited plants shoot length increment between 19.2 % and 41.3 % compared to the un-inoculated controls in 90 days after planting. The dual inoculation treatments consisted of AU27 (*B. shewense*) + *Glomus* sp.1 and AU27 (*B. shewense*) + *Acaulospora* sp.1 increased the *E. brucei* shoot length by 41.2 % and 37.8 %, respectively in 90 DAP. Similarly, the microbial consortia comprised of AU27 + RG6 (*A. soli*) + *Acaulospora* sp.1 and AU27 + AU4 (*A. soli*) + *Glomus* sp.1 increased shoot length by 32.5 % and 29.6 %, respectively in the same time period. Similar study was conducted by Berza et al. [17] in the greenhouse conditions by using the same host plant and microbial inputs. These authors reported 113 % and 111 % increment in *E. brucei* shoot length as a result of dual inoculation with AU27 + *Glomus* sp.1 and AU27 + *Acaulospora* sp.1, respectively in the greenhouse condition in 90 DAP. In addition, inoculation of *E. brucei* with the consortia of AU27 +AU4 + *Glomus* sp.1 and AU27 + AU4 + *Glomus* sp.1 + *Acaulospora* sp.1 increased the plant shoot length by 130 % and 140 %, respectively [17]. Such a big difference in the shoot length between Berza et al. [17] and the present study could be due to the field conditions both inoculated microbial inputs and *E. brucei* might have faced unpredictable biotic and abiotic stresses. For example, competition, drought, herbivory [31,32], weeds induce stress compared to sterile greenhouse condition [33] and the greater aboveground herbivores in the field may directly remove the aboveground biomass and induce increased belowground growth [34], Drought in the field may also decrease plant growth, microbial growth and nutrient recycling rates [35].The other important point is the contribution of AMF in the field condition. As can be seen from the data, during all the field experiment periods, treatments comprised of AMF inoculations exhibited higher performance compared to rhizobacteria alone or their combinations. This could be associated to the fact that AMF play a vital role in absorbing and transporting important nutrients and water to the host plant beyond root depletion zones. Moreover, AMF contribute to the plant growth by alleviating abiotic and biotic stresses [17]. The inoculation effects were become visible after 60 days after planting. These 60 days might have served as time of adaptation in the field and time of competition with the indigenous soil microorganisms and, then after our inoculants began to provide the intended plant-beneficial traits. Moreover, these 60 days could be time of producing sufficient root biomass by *E. brucei* so as to form symbiotic association with rhizobia and AMF.

Both single microbe inoculation and the microbial consortia application enhanced the growth of a rose flower under greenhouse conditions [36]. However, the application of microbial consortia has several benefits over single inoculation for plants in the greenhouse or field conditions. Within a consortium, different members can provide traits lacking in others, leading to enhanced overall effects on plant growth improvement. For instance, consortia consisting of microbes that produce indole-3-acetic acid and solubilize inorganic phosphate were more efficient at growth promotion than the strains applied individually [[37,38]].Similar enhanced plant growth could be observed by using combinations like plant-growth promoting bacteria plus arbuscular mycorrhizal fungi [39] and drought-mitigating isolates plus nitrogen fixers [40]. Moreover, members within a consortium can facilitate the establishment and functioning of target strains through synergistic cooperation [41]. This interaction was also observed between plant growth promoting rhizobacteria and AMF, where plants could achieve a higher salt-stress resistance [42] and better organic phosphorous mineralization [43] compared to inoculate with either microorganism alone. Furthermore, interactions between inoculants and indigenous species are more likely mediated through quorum sensing and antibiotic release within bacterial consortia [44]. These aspects support using microbial consortia to achieve more stable and effective outcomes [44–46].

In this study, we observed that all inoculated treatments reduced the soil pH compared to the un-inoculated and un-planted treatments. A soil acidification can result due to nutrient cycling and decomposition as we applied organic green manures. Mineralization and oxidation of the organic nitrogen, carbon and sulfur release H^+, thus lowering soil pH. The organic matter decomposition causes also the release of CO_2 into the soil air, which when dissolved in soil water forms H_2CO_3, which in turn causes a decline in a soil pH. In addition, plant growth is another factor which causes localized soil acidification as a result of nutrient up take. Plants commonly take up nutrients from the soil solutions in the ionic form with a preference to cations over anions which also lead to cation reduction in the soil [47]. To counteract the effects of charge imbalance, plants release H^+ from roots to the rhizosphere, thereby lowering soil pH.

Moreover, plant roots naturally release organic acids as exudates, which cause soil acidification. However, the pH range 5.5–6.5 is optimal for plant growth as the availability of nutrients is optimal. This is also true for most soil microbes, in part because in this pH range plants grow well and produce more root exudates as a carbon source available for survival and multiplication of microbes. Some microbes have the ability to alter soil pH by acidifying their surroundings, as a way to outcompete other microbes [48].

In the present study, the green manure plants (*E. brucei*) inoculated with *B. shewense* (AU27) increased the soil total nitrogen content by 17 % compared to the un-inoculated controls. In addition, the green manure plants inoculated with AU27 + AU4, AU27 +AU4 +*Glomus*sp.1 and AU27 + RG6 + *Acaulospora* sp.1 increased the soil total nitrogen status each by 2.4 %. This improvement could be associated with the efficiency of symbiotic nitrogen fixation by *B. shewense* (AU27) and synergistic effect exerted on the host plant growth by AMF and plant growth promoting endophytic bacteria inoculated in the present study [17]. Mon and Oue [49]have reported the soil total nitrogen content increment between 15.7 % and 32.8 % after application of clover mulching compared to the initial soil nutrient status before the green manuring. Similarly, Zhong et al. [50] have also reported the highest total and ammonium nitrogen content after application of *Arachispintoi* as a green manure, whereas nitrate nitrogen was the highest in the *Chamaecrista rotundifolia* mulched soils. Furthermore, according to Ma et al. [51], legume plants green manures more markedly increased both nitrate nitrogen and hydrolysable nitrogen and the mean increment in the nitrate nitrogen was significantly greater under legume green manure treatments compared to the non-legume green manure treatment.

All the inoculated treatments in the present study exhibited improved soil available phosphorous level. The highest (97.4 %) improvement in soil available phosphorous status was recorded by green manure plants inoculated with AU27 + AU4 followed 54.3 % due to AU27 + RG6 inoculation compared to un-inoculated control plant used as a green manure. Similarly, AU27 + AU4 + *Glomus* sp.1 and AU27 + *Glomus* sp.1 inoculated plant green manures improved the soil phosphorous status by 37.1 % and 33.8 %, respectively compared to the soils received un-inoculated plants as a green manure. This higher phosphorous status in the *E. brucei* biomass could be probably attributed to the fact that the inoculated microbes AU27, RG6 and AU4 were phosphate solubilizers (Table 1). These organisms might have solubilized the soil insoluble phosphates and provide sufficient phosphorous to the host plants. The AMF might have also played significant role by absorbing and translocating solubilized phosphorous, water and other nutrients [17]. Similar field studies using different host plant by different authors have revealed similar improvement trend in soil available phosphorous when applied as legume green manures. For instance, Adekiya [52] has reported improved soil phosphorous due to application of *Acacia, leucaena* and *Gliricidia* green manures. In addition, Zhong et al. [50] have recorded significantly higher total phosphorous in legume green manure mulched soils compared to non-mulched soils.

All soils mulched with inoculated and un-inoculated *E. brucei* plants as green manures improved soil organic matter and organic carbon. Plants inoculated with AU27 + *Glomus* sp.1 improved soil organic matter by 11.4 %, followed by 10.2 % due to AU27 single inoculation compared to the soils received un-inoculated plants as a green manure. In addition, soils received *E. brucei* green manure plants inoculated with AU27 + *Glomus* sp.1 improved the soil organic carbon by 4.6 %, whereas those received AU27 inoculated mulches improved 3.6 % compared to soils received un-inoculated plants. The organic matter and carbon are products of effective and successful photosynthesis. The improvements in the organic matter and carbon content could be directly associated with the contributions by our inoculants to the *E. brucei* efficient photosynthesis. The inoculated microorganisms produced IAA, solubilized phosphate and AU27 fixed atmospheric nitrogen to support efficient photosynthesis. Therefore, their synergistic effects could have improved photosynthesis. Similar work by Adekiya [52] has exhibited increment in soil organic matter. The application of *Acacia, leucaena* and *Gliricidia* green manures increased soil organic matter compared to initial soil nutrients status before the application of green manures [52]. Moreover, Zhong et al. [50] have reported significantly a higher soluble soil organic carbon, total soluble soil carbon and soil organic matter in legume green manures compared to non-mulched soils. Cover cropping with legumes and rice straw mulch significantly increased soil organic carbon, total nitrogen and phosphorous [53].

5. Conclusions

In the present study, the experimental field was devoid of indigenous *E. brucei* nodulating rhizobia, which was revealed by lack of nodules on tested *E. brucei* plant roots. In the first 60 day after planting, inoculation treatments did not exhibit significant differences in plant shoot length among inoculated treatments and between inoculated and un-inoculated treatments. However, increment in plant shoot length was recorded after 90,120 and 150 days of planting, respectively compared to the un-inoculated plants. The soil organic matter content exhibited improvement in both inoculated and un-inoculated treatments. The majority of the inoculated treatments showed improvement in soil available phosphorous level. Legume plants inoculated with effective rhizobia, phosphate solubilizing rhizobacteria and AMF can improve the soil nitrogen and phosphorous in the field conditions.

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CRediT authorship contribution statement

Belay Berza Beyene: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fassil Assefa Tuji:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30484.

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