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Bacillus PGPR consortium as a partial substitute for poultry litter improves forage performance in annual ryegrass and tall fescue under greenhouse conditions

Felix Satognon[1*], Dexter B. Watts[2], Anthony O. Adesemoye[2], H. Allen Torbert[2], Rishi Prasad[1] and Mary Kimberly Mullenix[3]

*Correspondence:
Felix Satognon
fzs0030@auburn.edu
[1]Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL, US
[2]USDA-ARS National Soil Dynamics Laboratory, Auburn, AL, US
[3]Department of Animal Sciences, Auburn University, Auburn, AL, US

Abstract

Excessive poultry litter (PL) application in forage systems poses environmental risks. This study evaluated the potential of plant growth-promoting rhizobacteria (PGPR) to reduce PL dependence in annual ryegrass (*Lolium multiflorum*) and tall fescue (*Lolium arundinaceum*) production. Four PL rates (0%, 50%, 75%, 100% of 168 kg N/ha) were tested with and without a bacillus-based PGPR consortium (*B. altitudinis* AP18, *B. subtilis* AP278, *B. safensis* AP280, and *Lysinibacillus macroides* AP282) in a randomized complete block design with six replicates. PGPR had no significant effect on seedling emergence but significantly enhanced plant height, tiller number, shoot and root biomass, and root traits across PL rates. These impacts were more pronounced in annual ryegrass. Ryegrass under 50% and 75% PL + PGPR was 3.8% and 8.66% taller than 100% PL alone. Tall fescue under 75% PL + PGPR was 2.84% taller than 100% PL, while 50% PL was slightly shorter but not significant. Tiller numbers under 50–75% PL + PGPR matched those of 100% PL in both grasses. Shoot biomass at 50% and 75% PL + PGPR in ryegrass (2.23 and 2.18 g/container) and tall fescue (1.08 and 0.44 g/container) was similar to 100% PL (2.02 and 0.44 g/container). PGPR at 50–75% PL increased root biomass by 10–12% in ryegrass and 4–17% in tall fescue. Additionally, 50–75% PL + PGPR improved root traits in the 0.51–2.00 mm and > 2 mm diameter classes compared to 100% PL. PGPR with reduced PL supports sustainable forage production.

Keywords Plant growth-promoting rhizobacteria, Poultry litter, Root architecture, Sustainable forage production, Forage yield

1 Introduction

Continuous application of both organic and inorganic fertilizers including poultry litter (PL) has become indispensable in both conventional and organic farming [1]. In the United States the use of PL is widely adopted in different farming system due to the high cost of agricultural inputs including synthetic fertilizers. PL is widely used as a key

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nutrient source in the southeastern United States, where poultry farming is the leading industry [2]. Historically PL application has been reported to improve low-fertility, acidic, and shallow soils, particularly in mountainous regions of the southeastern United States, by supplying essential nutrients and liming elements like calcium and magnesium, thereby enhancing productivity in pastures and hayfields [2, 3]. Unfortunately, continuous or excess application can have a negative impact on the environment [4]. For instance, continuous applications of PL can cause nutrient accumulation in soils, leading to runoff that contaminates nearby water bodies [1, 5]. This nutrient-rich runoff often triggers excessive algal growth in surface waters, degrading water quality and, in some cases, prompting legal action when municipal water supplies are impacted [2]. Another challenge with using PL is its slow nutrient release, which can lead to poor synchronization with crop nutrient demand at specific growth stages. This mismatch may worsen environmental issues, reduce crop response to PL applications, and ultimately lower farm income [6, 7].

Biostimulants and biofertilizers emerge as sustainable, and cost-effective technologies that can play a key role in addressing environmental challenges resulting from excessive use of agrochemicals in modern agriculture [8, 9]. These products, containing beneficial microorganisms such as bacteria, fungi, and algae, support soil health while enhancing water and nutrients uptake, nutrient cycling and plant growth [10]. In addition to improving yields, they reduce reliance on organic and inorganic agricultural inputs and provide plants with essential nutrients and growth-promoting hormones [11, 12]. These biofertilizers were also reported to improve crop quality, stress tolerance, soil microbial communities, and act as biocontrol agents [9, 11, 12].

Biofertilizers are formulated using various living microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), which are known for their ability to enhance plant growth and soil health. PGPRs include diverse bacterial genera such as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Azotobacter*, *Paenibacillus*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, *Frankia*, *Enterobacter*, *Bradyrhizobium*, *Flavobacterium*, *Allorhizobium*, and others [9, 13]. These free-living bacteria inhabit the rhizosphere and support plant growth through both direct and indirect mechanisms. Directly, PGPR promote plant growth by fixing atmospheric nitrogen and solubilizing essential soil nutrients [14, 15]. PGPR also modulate phytohormones production in the plant like auxins, cytokinins, abscisic acid, gibberellins, and ethylene, thereby influencing root and shoot development, and stress responses [16]. Indirectly, PGPR acts in suppressing phytopathogens and other harmful microorganisms, contributing to plant protection and improved resilience [17–19]. Together, these mechanisms make PGPR valuable tools for sustainable agriculture. Bacillus-based biofertilizers are generally more active than pseudomonas-based ones due to their efficient metabolite production and spore-forming capacity, which enhance cell viability in commercial formulations [20, 21]. *Bacillus* spp., gram-positive bacteria, ubiquitous in nature, and found in all environmental niches, have been widely used in biofertilizer formulations [22]. These beneficial bacteria associate with plant roots or rhizospheres, forming biofilms that support plant growth and offer a sustainable alternative to chemical fertilizers and pesticides [20, 23]. For instance, a biofilm-forming *Bacillus subtilis* strain from Brazil enhanced growth of corn (*Zea mays*) and wheat (*Triticum aetivum*) by solubilizing phosphorus in phosphorus deficient soils [24]. These biofilms not only improve nutrient availability, through nitrogen

fixation and organic acid production, but also produce antimicrobial compounds and trigger systemic resistance to protect plants from pathogens [25]. As a result, bacillus-based biofertilizers support plant health by enhancing nutrient access and activating pest and disease defense mechanisms in the rhizosphere [26].

Although numerous studies in the literature have reported the positive effects of PGPR on plant growth and yield, most have focused on row crops [27–34], with limited research dedicated to forage production systems [35–38]. Furthermore, the practical application of PGPR to reduce reliance on both organic and inorganic fertilizers remains largely underexplored in forage systems, despite its potential to mitigate environmental pollution and support sustainable forage production. Forage production is an important sector of the Southern United States, where it supports the region's strong livestock industry [39]. The favorable climate of the Southern United States allows for the successful cultivation of a wide range of forage species, with annual ryegrass and tall fescue being the predominant cool-season grasses. Tall fescue is a deep-rooted, perennial C3 bunchgrass that responds well to nitrogen fertilization, while annual ryegrass is a C3 annual commonly used to overseed warm-season perennial pastures [40]. This practice extends the grazing season, particularly during forage-deficient periods [41]. Although various nutrient management practices have been developed for these species, limited research has explored the combined use of PGPR and PL as a strategy for sustainable forage production. This study evaluated the potential of bacillus-based PGPR as a partial substitute for PL in the cultivation of annual ryegrass and tall fescue under greenhouse conditions, with the hypothesis that PL inputs could be reduced by 25–50% without significantly compromising forage yield.

2 Materials and methods

2.1 Greenhouse location, temperature and relative humidity

Two separate container experiments were conducted under greenhouse conditions between February and April 2025 at the USDA-ARS National Soil Dynamics Laboratory in Auburn, Alabama, United States. The climatic conditions within the greenhouse such as the temperature and relative humidity collected with data logger every 20 min are summarized in Fig. 1.

2.2 Collection and analysis of soil and poultry litter

Soil used in this experiment was collected during the Fall of 2024 from E.V. Smith Research Center of Auburn University located in Shorter, Alabama. To ensure sample

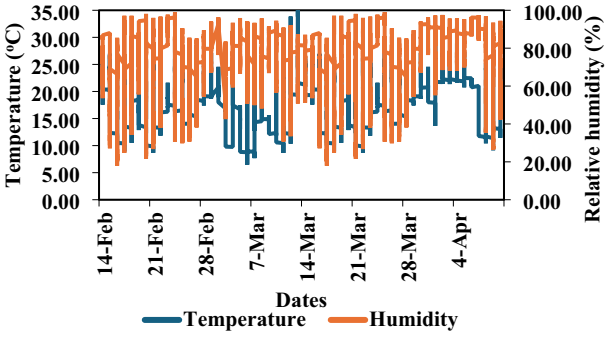


Fig. 1 Climatic conditions (temperature and relative humidity) within the greenhouse

Table 1 Physical and chemical properties of soil and PL used in this experiment

Parameters	Soil	PL
Dry/wet (%)	-	90.2 (Dry Matter)
Ash (%)	-	20.1
Carbon (%)	-	79.93
Organic matter (%)	1.3	-
Sand (%)	47.5	-
Silt (%)	46.25	-
Clay (%)	6.25	-
Soil texture	Sandy loam	-
Nitrogen (%)	0.036	3.25
Phosphorus (%)	0.00152	1.77
Potassium (%)	0.0059	2.82
Calcium (%)	0.0274	3.16
Magnesium (%)	0.00947	0.66
Aluminum (mg/kg)	103	584
Boron (mg/kg)	0.3	52
Copper (mg/kg)	1	74
Iron (mg/kg)	15.4	766
Manganese (mg/kg)	104	733
Sodium (mg/kg)	44.5	9110
Zinc (mg/kg)	3.2	511
Cation exchange capacity (meq/100 g)	3.47	-
pH	4.95	6.9

uniformity, soil collected from the upper 15 cm from various points was combined, air-dried, and sieved through a 2 mm mesh. PL was collected from a nearby commercial poultry facility in Auburn, Alabama, United States. Both soil and litter samples underwent laboratory analysis at Auburn University's Soil Testing Laboratory. Soil texture, specifically the proportions of sand, silt, and clay, was determined using the hydrometer method as described by Bouyoucos [42]. Chemical properties of both the soil and the poultry litter were evaluated using the analytical procedures outlined by Hue and Evans [43]. The physical and chemical properties of soil and PL used in this experiment are summarized in Table 1.

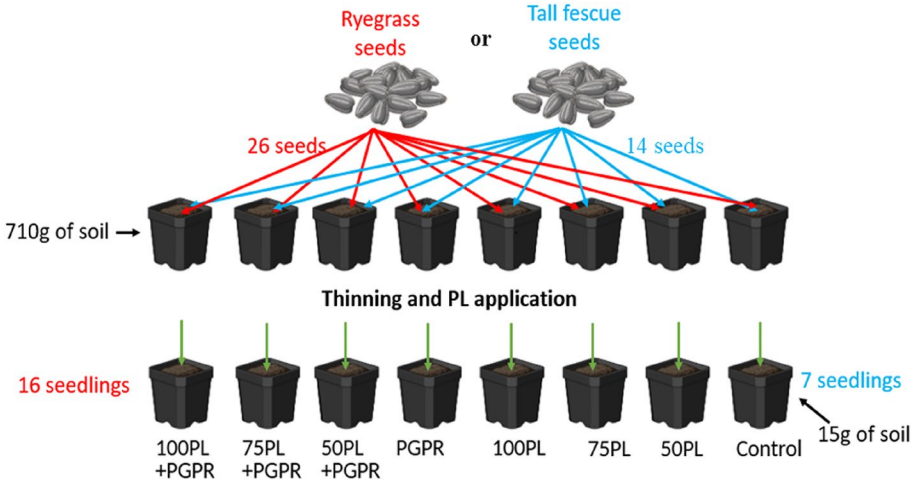


Fig. 2 Experimental layout

2.3 Experimental setup and treatments

The two greenhouse experiments were conducted using 5-inch Azalea nursery containers (12 cm top diameter, 8 cm bottom diameter, 9 cm height), each filled with 710 g of non-sterile air-dried, 2 mm-sieved soil (Fig. 2). All containers were watered to 75% of their water holding capacity (WHC) before planting. To determine WHC, three containers filled with the same amount of soil were saturated with water until it drained from the bottom. The average water volume required for saturation was recorded and used as the 100% WHC value. Certified seeds of tall fescue (Texoma MaxQ II), a novel endophyte-infected cultivar released by the Samuel Roberts Noble Foundation in 2011, and annual ryegrass (Rapido), obtained from Southeast AgriSeeds, LLC (USA), were sown at rates of 14 seeds per container for tall fescue and 26 seeds per container for annual ryegrass. After sowing, an additional 15 g of soil was added to cover the seeds. The experiment consisted of eight treatments designed to evaluate the effects of PL reduction and PGPR inoculation on forage grasses. The treatments were as follows:

1. 100% PL
2. 100% PL + PGPR consortium
3. 75% PL
4. 75% PL + PGPR consortium
5. 50% PL
6. 50% PL + PGPR consortium
7. 0% PL
8. 0% PL + PGPR consortium

The PGPR microbial suspension was inoculated at sowing using pipette at a rate of 10 mL per container, delivering approximately 1.5×10^7 colony-forming units (CFU)/mL. Seven days after sowing, seedlings were thinned to maintain 8 plants per container for tall fescue and 16 plants per container for annual ryegrass, corresponding to seeding rates of 10 kg/ha and 30 kg/ha, respectively. PL was applied immediately after thinning, followed by an additional 15 g of soil to cover the litter, bringing the total soil weight to 740 g/container. The 100% PL treatment corresponded to 168 kg total N/ha, with application rates calculated based on a 15 cm soil depth over one hectare (approximately 2,000,000 kg of soil) [44]. The actual amounts of PL applied per container for the 0%, 50%, 75%, and 100% PL treatments were 0, 1, 1.5, and 2 g, respectively. All treatments were arranged in a randomized complete block design (RCBD) with six replicates. Soil moisture was maintained at approximately 75% WHC throughout the study using an automated sprinkler irrigation system. Forage grasses were harvested six weeks after sowing. The experiment was repeated twice. The second experimental run was set up three weeks after the first experiment.

2.4 Preparation of inoculum

The microbial suspension used for PGPR inoculation consisted of a consortium of four *Bacillus* strains, selected based on evidence that multi-strain formulations are generally more effective than single-strain inoculants for enhancing plant growth [45, 46]. The bacteria used in the consortium for this study were obtained from the culture collection of Auburn University and were previously characterized and reported with multiple traits, including enhancing the growth and yield of corn (*Zea mays*) [47]; and

colonization, growth promotion, and biological control of grasses [47–49]. The strains included:

1. *Bacillus altitudinis* AP18
2. *Bacillus subtilis* AP278
3. *Bacillus safensis* AP280
4. *Lysinibacillus macroides* AP282

To prepare the inoculum, each strain was aseptically revived from − 80 °C glycerol stocks by streaking onto tryptic soy agar (TSA) plates and incubating for 24–36 h. A loopful of each culture was then inoculated into 250 mL of tryptic soy broth (TSB) in 500 mL Pyrex bottles and grown in an orbital incubator shaker (SHKE4000, Thermo Fisher Scientific Inc., USA) at 130 rpm and 27 °C for 12–14 days. Following incubation, cultures were centrifuged using 50 mL Falcon tubes in a Sorvall X1 Pro-MD centrifuge (Thermo Fisher Scientific Inc., USA) at 5,500 × g for 15 min to concentrate the bacterial cells. The supernatant was discarded, and the pellets were re-suspended in sterile distilled water. The final bacterial suspension was adjusted to a concentration of approximately 1.5×10^7 colony-forming units (CFU)/mL. Equal volumes of each strain were combined to create the final PGPR consortium inoculum.

2.5 Evaluation of plant growth and yield parameters

Seedling emergence was monitored for seven days following sowing. Plant height was recorded at both two weeks and six weeks (harvest) using a standard ruler. At harvest, plants with the soil were gently removed from the containers, thoroughly washed, and tillers were counted. Plants were then separated into shoot and root fractions. Roots were stored at 4 °C until further analysis using the WinRHIZO root scanning system (Regent Instruments Inc., Canada). Prior to scanning, roots were carefully rinsed to remove residual soil particles and spread evenly in a 30 × 40 cm transparent tray filled with 300 mL of water to reduce overlap and ensure accurate measurement. Roots were scanned at high resolution to quantify total root length, surface area, average diameter, volume, number of tips and forks, and distribution by diameter class. Post-scanning, root and shoot samples were dried at 55 °C until a constant weight, and dry biomass was recorded for each component. The root-to-shoot biomass ratio was subsequently calculated.

2.6 Data analysis

All statistical analyses were performed using R software [50]. Prior to conducting parametric tests, data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. A mixed-effects analysis of variance (ANOVA) was used to evaluate treatment effects, with block and experimental run included as random effects to account for spatial and temporal variability. When significant differences among treatments were detected ($p < 0.05$), the means were separated using the Least Significant Difference (LSD) test at a 5% significance level. The statistical analyses and visualizations were conducted using a suite of R packages, including car, emmeans, ggplot2, dplyr, agricolae, patchwork, tidyr, lme4, lmerTest, and multcomp [51–57].

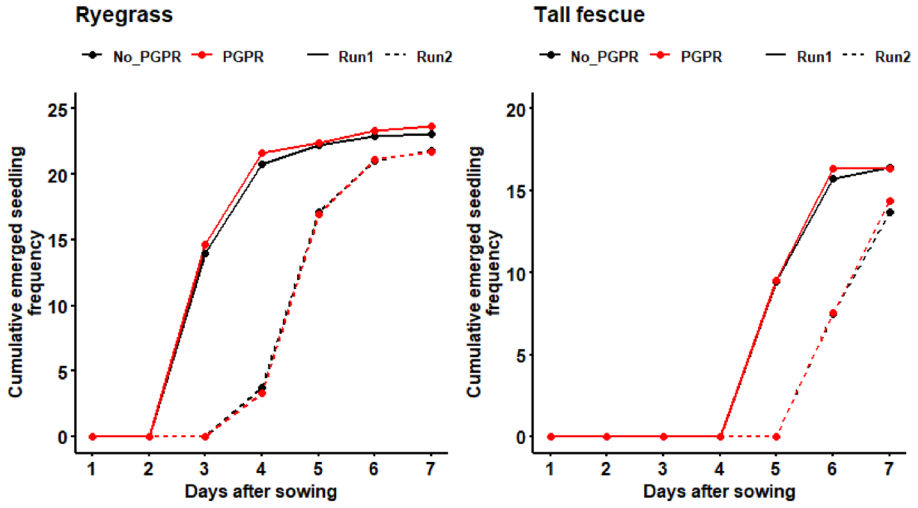


Fig. 3 Effect of PGPR inoculation on cumulative emerged seedlings of annual ryegrass and tall fescue

Table 2 P-values showing the significance of the effects of PGPR inoculation, PL, grass species, and their interaction on plant height and number of tillers

	Height at 2 weeks	Height at 6 weeks	Number of tillers
PL	**	***	***
PGPR	***	***	***
Grass species	***	***	***
PL×PGPR	ns	ns	ns
PL×Grass species	ns	ns	***
PGPR×Grass species	ns	ns	ns
PL×PGPR×Grass species	ns	ns	ns

Significance levels: *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), *ns* not significant

3 Results

3.1 Seedling emergence

The seedling emergence frequency from the soil of both annual ryegrass and tall fescue seeds were not significantly influenced by PGPR inoculation (Fig. 3). However, a delay in in seedling emergencewas observed in the second experiment, primarily due to low greenhouse temperatures at the beginning of the experiment. Comparable seedling emergence frequency was recorded for both PGPR-inoculated and non-inoculated treatments throughout the experiment.

3.2 Growth parameters

The effects of PL, PGPR, grass species, and their interactions on plant height and tiller number are presented in Table 2. Overall, annual ryegrass plants were taller than tall fescue plants two weeks after sowing across all treatments. However, by six weeks after sowing, the growth pattern varied between experiments. In the first experiment, tall fescue plants were slightly taller than annual ryegrass, whereas in the second experiment, annual ryegrass plants were taller than tall fescue. The impact of PGPR on annual ryegrass height was consistent across different PL levels, with the most pronounced effects observed at 75% PL and 50% PL (Fig. 4). PGPR inoculation consistently enhanced ryegrass height across PL levels, with height increases observed at all PL rates. For example, at 6 weeks PGPR treatments showed a height increase of roughly 5–11% across PL rates,

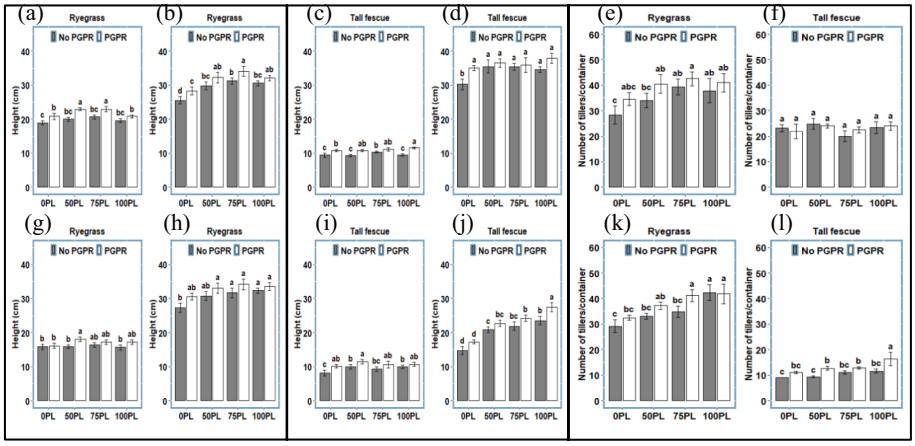


Fig. 4 Effect of PGPR and PL on plant height and tiller number of annual ryegrass and tall fescue. **a** and **g** show annual ryegrass height at 2 weeks after sowing; **b** and **h** show annual ryegrass height at 6 weeks; **c** and **i** show tall fescue height at 2 weeks; **d** and **j** show tall fescue height at 6 weeks; **e** and **k** show the number of tillers in annual ryegrass; **f** and **l** show the number of tillers in tall fescue. **a**–**f** are from experimental run 1, while (**g**–**l**) are from run 2. Different letters indicate significant differences at $p < 0.05$. 0PL, 50PL, 75PL and 100PL correspond to 0% PL, 50% PL, 75% PL, 100% PL, respectively

including a 9.5% increase at 75% PL. When comparing PGPR treatments at reduced PL rates to the 100% PL only treatment, ryegrass at 50%, 75% and 100% PL was about 3.8%, 8.66% and 4.53% taller than the 100% PL without PGPR, respectively indicating that PGPR can help maintain or even improve growth at reduced PL rate.

The positive influence of PGPR also extended to tall fescue height. The impact of PGPR and PL on tall fescue plant height was more pronounced at 6 weeks after sowing, specifically in experimental run 2. At 6 weeks, PGPR enhanced plant height by 16.9% at 0% PL, 9.5% at 50% PL, 10.5% at 75% PL, and 16.6% at 100% PL than non-inoculated plants. Heights at 75% PL with PGPR were about 11.7% lower than 100% PL with PGPR at 6 weeks. The 100% PL treatment with PGPR typically produced the tallest plants, except for measurements taken at 2 weeks in experimental run 2. However, the height of the plant under 100PL with no PGPR inoculation was not significantly different from the heights recorded in the 50% PL and 75% PL treatments with PGPR. When comparing PGPR treatments at reduced PL rates to the 100% PL with no PGPR, heights were 3.31% lower and 2.84% higher under 50% and 75% PL, respectively. At the same time plants grown with 100PL with PGPR were 13.0% more taller than 100% PL only. The shortest plants were consistently observed in the 0PL treatment without PGPR. PGPR also influenced the number of annual ryegrass tillers/container across different PL rates. While the number of tillers generally increased with an increment in PL, PGPR inoculation further enhanced them. PGPR inoculation increased the number of tillers of annual ryegrass by up to 21.8% at 0% PL, 19.12% at 50% PL, 8.0% at 75% PL and about 8.0% at 100% PL compared to their irrespective non-inoculated treatments. The highest tiller counts were recorded in the 100% PL and 75% PL treatments with PGPR, though these were statistically similar to 50% PL with PGPR. Additionally, the number of tillers, 40 and 42, respectively in 50% PL and 75% PL with PGPR matched those in 100% PL without PGPR (41). For tall fescue, treatment effects on the number of tillers were only significant in the second experimental run. The 100% PL treatment with PGPR produced the highest number of tillers (16), while 50% PL (12) and 75% PL (13) with PGPR had similar number of tillers compared to 100% PL alone (12).

Table 3 P-values showing significance of the effects of PGPR inoculation, PL, grass species, and their interaction on shoot and root dry biomass and root to shoot ratio

	Shoot dry biomass	Root dry biomass	Root to shoot ratio
PL	***	**	***
PGPR	***	*	ns
Grass species	***	***	***
PL×PGPR	*	ns	ns
PL×Grass species	*	ns	ns
PGPR×Grass species	*	ns	ns
PL×PGPR×Grass species	ns	ns	ns

Significance levels: *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), *ns* not significant

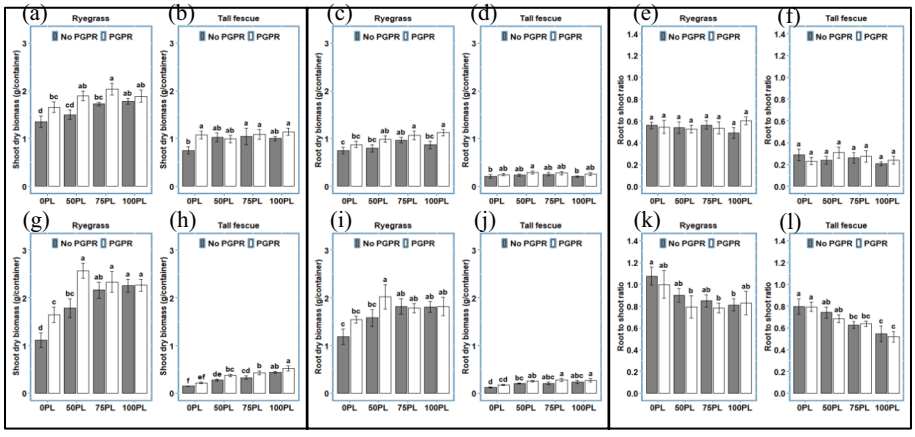


Fig. 5 Effect of PGPR and PL on shoot and root dry biomass and root to shoot ratio of annual ryegrass and tall fescue. **a** and **g** show annual ryegrass shoot dry biomass; **b** and **h** show tall fescue shoot dry biomass; **c** and **i** annual ryegrass root dry biomass; **d** and **j** show tall fescue root dry biomass; **e** and **k** annual ryegrass root to shoot ratio; **f** and **l** show tall fescue root to shoot ratio. **a**–**f** are from experimental run 1, while (**g**–**l**) are from run 2. Different letters indicate significant differences at $p < 0.05$. 0PL, 50PL, 75PL and 100PL correspond to 0% PL, 50% PL, 75% PL, 100% PL, respectively

3.3 Dry biomass and root to shoot ratio

Poultry litter, PGPR, grass species and their interactions except PL×PGPR×grass species significantly affected the shoot dry biomass (Table 3). Generally, annual ryegrass exhibited higher shoot and root biomass compared to tall fescues (Fig. 5). The shoot dry biomass of ryegrass generally increased with PL rates. However, PGPR inoculation further enhanced shoot dry biomass at different PL rates. For instance, PGPR inoculation increased shoot dry biomass of annual ryegrass by 35.5% at 0% PL, 35.2% at 50% PL, 13.9% at 75% PL and about 3.1% at 100% PL. The highest shoot dry biomass was observed in 75% PL + PGPR and 50% PL + PGPR in experimental runs one and two, respectively. In addition, the shoot dry biomass under 75% PL + PGPR (2.18 g/container) and 50% PL + PGPR (2.23 g/container) was statistically similar to that recorded under 100% PL with (2.08 g/container) or without PGPR (2.02 g/container). Inoculation of PGPR at 100% PL yielded statistically similar shoot dry biomass as 100% PL alone, indicating less benefit at higher PL levels. The shoot dry biomass of tall fescue was also significantly affected by PGPR and PL application (Fig. 5). During the first experimental run, all treatments exhibited higher shoot dry biomass compared to 0PL with no PGPR. However, in the second experimental run, though less dry biomass was observed compared to the first run, shoot dry biomass increased with PL increment. PGPR inoculation

enhanced tall fescue shoot dry biomass at each PL level. Across the two experimental runs, PGPR inoculation increased the shoot dry biomass of tall fescue by 44.4% at 0% PL, 16.2% at 50% PL, 16.6% at 75% PL and about 13.7% at 100% PL. The maximum shoot dry biomass was observed in 100% PL + PGPR (1.14 and 0.52 g/container in runs 1 and 2, respectively). Interestingly, 75% PL with PGPR exhibited similar dry biomass yield (1.08 and 0.44 g/container, respectively) compared to 100% PL with or without PGPR inoculation (1.00 and 0.44 g/container, respectively).

The root dry biomass of annual ryegrass was higher under all PL treatments compared to 0% PL. PGPR inoculation further enhanced root biomass by approximately 10–29% across various PL rates, with the most notable increases observed in the first experimental run. In the second run, PGPR application increased root biomass by 30.6% at 0% PL and by 28.1% at 50% PL. Additionally, the 50% PL and 75% PL treatments with PGPR produced approximately 10% and 12% higher root biomass, respectively, compared to the 100% PL-only treatment (0.97 g/container) across both experimental runs. A similar positive effect of PGPR and PL was observed on the root dry biomass of tall fescue. In the first experimental run, the highest root dry biomass was recorded under the 50% PL + PGPR treatment (0.29 g/container), although this value was only significantly different from the 0% PL and 100% PL treatments without PGPR. In the second run, the maximum root dry biomass was observed under the 75% PL and 100% PL treatments with PGPR inoculation. Notably, the 50% PL and 75% PL treatments with PGPR also produced root biomass values (0.25 and 0.28 g/container in runs 1 and 2, respectively) that were comparable to the 100% PL treatment (0.24 g/container).

The root-to-shoot ratio of both grasses was not significantly affected in the first experimental run. However, in the second run, the root-to-shoot ratio decreased with increasing PL rates, regardless of PGPR inoculation. PGPR application at 50% PL slightly reduced the root-to-shoot ratio of annual ryegrass at 0%, 50%, and 75% PL, although the differences were not statistically significant when compared to their respective non-inoculated treatments. Interestingly, the root-to-shoot ratio under the 75% PL + PGPR treatment was statistically similar to that observed under the 100% PL treatment for both grasses.

3.4 Root traits

Poultry litter, PGPR, and grass species significantly influenced root traits in both annual ryegrass and tall fescue, as detailed in Table 4. In addition, the interaction between PL and grass species was significant for root surface area, volume, and number of forks, while the interaction between PGPR and grass species was significant for average root diameter, volume, and number of forks. However, the interactions between PL × PGPR and PL × PGPR × grass species were not significant for any of the root traits. Overall, root traits improved with increasing PL application rates up to 75%, beyond which no additional benefits were observed at 100% PL. Annual ryegrass exhibited a stronger response to both PL and PGPR treatments compared to tall fescue. PGPR inoculation consistently enhanced root length across all PL levels. In annual ryegrass, the combination of 50% PL + PGPR increased root length by 5.9% in experimental run 1 and by 13.9% with 75% PL + PGPR compared to 100% PL without PGPR (16,435 cm vs. 14,416 cm, respectively). In run 2, root length was 6.4% higher with 50% PL + PGPR and 5.4% higher with 75% PL + PGPR. For tall fescue, the response was even more pronounced. In run 1,

Table 4 Effect of PGPR and PL litter on root length, surface area, average diameter, volume, number of tips and number of forks of annual ryegrass and tall fescue

PL	PGPR	Length (cm)	Surface area (cm2)	Average diameter (mm)	Volume (cm3)	Number of tips	Number of forks
Annual ryegrass							
Run 1							
0% PL	No PGPR	13576.109b	995.106c	0.231b	11.517 g	106954b	162453c
0% PL	PGPR	14018.594ab	1050.943b	0.239ab	12.756f	114330ab	176656bc
50% PL	No PGPR	14319.759ab	1067.062b	0.241ab	13.517ef	117308ab	182329bc
50% PL	PGPR	15266.821ab	1236.393a	0.258a	17.492b	124766ab	198626ab
75% PL	No PGPR	14794.071ab	1172.269a	0.252ab	14.366d	113451ab	193711abc
75% PL	PGPR	16434.974a	1340.963a	0.260a	19.105a	137015a	227074a
100% PL	No PGPR	14416.397ab	1143.899a	0.253ab	16.039c	114172ab	188449bc
100% PL	PGPR	16088.900a	1261.165a	0.260a	16.846c	130420ab	200429ab
Run 2							
0% PL	No PGPR	17035.115c	1190.225d	0.201c	12.402e	136839d	200598d
0% PL	PGPR	18766.357bc	1372.368d	0.222b	15.788d	159,585 cd	242,066 cd
50% PL	No PGPR	19717.027abc	1499.220b	0.233b	19.212c	164625bcd	260882bcd
50% PL	PGPR	22822.943a	1822.905a	0.252ab	26.372a	210697a	345193a
75% PL	No PGPR	20987.223ab	1696.342ab	0.250ab	24.773a	179100bc	285308abc
75% PL	PGPR	22605.697a	1740.778ab	0.237ab	22.897b	189163ab	309598a
100% PL	No PGPR	21450.563ab	1637.012ab	0.228b	20.665bc	179031bc	285799abc
100% PL	PGPR	20726.237ab	1712.370ab	0.268a	26.188a	184735abc	303414abc
Tall fescue							
Run 1							
0% PL	No PGPR	4334.595c	308.160b	0.205a	3.015b	42559ab	39517bc
0% PL	PGPR	4882.078b	346.922ab	0.208a	3.488ab	45815ab	44348abc
50% PL	No PGPR	4923.567b	359.028ab	0.215a	3.588ab	43856ab	44043abc
50% PL	PGPR	5556.287a	395.805a	0.210a	4.083a	50374a	52169ab
75% PL	No PGPR	4800.702ab	356.690ab	0.218a	3.783ab	41712ab	46464abc
75% PL	PGPR	5633.078a	396.118a	0.207a	3.860a	49167a	53765a
100% PL	No PGPR	4384.643ab	321.258ab	0.215a	3.257ab	39224b	38459c
100% PL	PGPR	5104.558ab	372.212ab	0.212a	3.768ab	47335ab	48428abc
Run 2							
0% PL	No PGPR	2979.255d	179.572d	0.173b	1.472e	23785d	20956d
0% PL	PGPR	4056.458c	243.703c	0.175b	1.903d	32110c	28874c
50% PL	No PGPR	4222.82bc	272.437bc	0.188a	2.390 cd	36880abc	34591b
50% PL	PGPR	5069.58ab	325.015ab	0.185a	2.797abc	42860ab	41873ab
75% PL	No PGPR	4460.603bc	286.888bc	0.187a	2.505bcd	35762bc	36128bc
75% PL	PGPR	5505.872a	356.718a	0.188a	3.038ab	40879ab	44003ab
100% PL	No PGPR	4907.115abc	321.95ab	0.190a	2.878abc	38123abc	39984ab

Table 4 (continued)

PL	PGPR	Length (cm)	Surface area (cm2)	Average diameter (mm)	Volume (cm3)	Number of tips	Number of forks
100% PL	PGPR	5731.193a	375.032a	0.190a	3.257a	43455a	46335a
P-values							
PL		***	***	***	***	***	***
PGPR		**	**	*	**	**	**
Grass species		***	***	***	***	***	***
PL×PGPR		ns	ns	ns	ns	ns	ns
PL×Grass species		ns	*	ns	***	ns	*
PGPR×Grass species		ns	ns	*	*	ns	*
PL×PGPR×Grass species		ns	ns	ns	ns	ns	ns

Different letters within a column indicate significant differences at $p < 0.05$. Significance levels: *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), *ns* not significant

root length increased by 26.7% with 50% PL + PGPR and 28.5% with 75% PL + PGPR. In run 2, the increases were 3.3% and 12.2%, respectively. Surface area followed a similar pattern. In annual ryegrass, 50% PL + PGPR increased surface area by 8.1% in run 1 and 11.7% in run 2, while 75% PL + PGPR resulted in increases of 17.2% and 6.3%, respectively. In tall fescue, the surface area improved by over 23% with 50% PL + PGPR and 75% PL + PGPR in run 1. In run 2, the surface area was 1.0% higher with 50% PL + PGPR and 10.8% higher with 75% PL + PGPR. In annual ryegrass, the greatest average root diameter was recorded under 100% PL + PGPR, also 50% PL + PGPR and 75% PL + PGPR yielded larger diameters than 100% PL without PGPR. For tall fescue, treatment effects were minimal in run 1, but in run 2, all PL treatments showed higher average diameters than 0PL, regardless of PGPR application.

Root volume exhibited the strongest response, particularly in annual ryegrass. In run 1, 50% PL + PGPR increased volume by 9.1%, while 75% PL + PGPR resulted in a 19.1% increase compared to 100% PL without PGPR. In run 2, volume increased by 27.7% with 50% PL + PGPR and 10.8% with 75% PL + PGPR. For tall fescue, 50% PL + PGPR increased volume by 25.4% in run 1, while 75% PL + PGPR led to an 18.5% increase. In run 2, 50% PL + PGPR improved volume by 5.2%, though 75% PL + PGPR resulted in a 7.6% reduction. PGPR consistently improved root branching traits, such as the number of tips and forks. In annual ryegrass, 50% PL + PGPR increased the number of tips by 15.3% and forks by 15.9% in run 1, while 75% PL + PGPR boosted these metrics by 20.0% and 20.5%, respectively. In run 2, 50% PL + PGPR produced 17.7% more tips and 20.7% more forks.

For tall fescue, the number of forks increased by 35.6% with 50% PL + PGPR and 35.7% with 75% PL + PGPR in run 1. In run 2, the number of tips rose by 12.4% and 13.7%, respectively, while the number of forks increased by 4.7% and 15.8%. These improvements highlight PGPR's role in enhancing the root system, particularly under moderate PL levels.

3.5 Root diameter class distribution

PL, PGPR inoculation, and grass species significantly influenced root length, surface area, and volume across all root diameter classes, with the strongest effects observed in 0.51–2.00 mm and > 2 mm root classes (Tables 5 and 6). While interactions between PL and PGPR were not significant, the interactions between PL × grass species and

Table 5 P-values showing significance of the effects PGPR inoculation, PL, grass species, and their interaction on root length, surface area, and volume of annual ryegrass and tall fescue across root diameter classes

	0–0.25 mm	0.26–0.50 mm	0.51–2.00 mm	> 2 mm
Length				
PL	**	**	***	***
PGPR	**	*	**	*
Grass species	***	***	***	***
PL×PGPR	ns	ns	ns	ns
PL×Grass species	ns	ns	**	**
PGPR×Grass species	ns	ns	ns	**
PL×PGPR×Grass species	ns	ns	ns	**
Surface area				
PL	***	***	***	***
PGPR	**	*	**	*
Grass species	***	***	***	***
PL×PGPR	ns	ns	ns	ns
PL×Grass species	ns	ns	**	**
PGPR×Grass species	ns	ns	*	*
PL×PGPR×Grass species	ns	ns	ns	ns
Volume				
PL	***	***	***	***
PGPR	**	*	**	*
Grass species	***	***	***	***
PL×PGPR	ns	ns	ns	ns
PL×Grass species	ns	ns	**	**
PGPR×Grass species	ns	ns	*	*
PL×PGPR×Grass species	ns	ns	ns	ns

Significance levels: *** ($p \leq 0.001$), ** ($p \leq 0.01$), * ($p \leq 0.05$), *ns* not significant

PGPR × grass species became significant in the larger root diameter classes. In both species, the 0–0.25 mm class comprised the largest proportion of root length and surface area but showed minimal response to PL and PGPR. The most responsive classes were 0.51–2.00 mm and > 2.00 mm. In annual ryegrass, root length in the 0.51–2.00 mm class increased by 9.2% with 50% PL + PGPR and 21.7% with 75% PL + PGPR in run 1, while the > 2.00 mm class saw increases of 19.4% and 33.8%, respectively. Surface area and volume followed similar trends, with particularly strong improvements in the > 2.00 mm class. In run 2, responses were even more pronounced. Root length in the 0.51–2.00 mm class increased by 14.5% with 50% PL + PGPR and 8.1% with 75% PL + PGPR, while the > 2.00 mm class saw a 44.8% increase under 50% PL + PGPR compared to 100% PL with no PGPR. Surface area and volume also showed substantial gains. For tall fescue, root traits across diameter classes were generally less responsive. The 0.51–2.00 mm class showed measurable improvements, particularly in run 1, where root length increased by 1.6% with 50% PL + PGPR and 15.9% with 75% PL + PGPR. Root volume in this class increased by 22.2% and 20.0%, respectively. In run 2, 50% PL + PGPR increased root length by 13.5%, surface area by 7.5%, and volume by 13.3%, while 75% PL + PGPR yielded more modest gains. Root traits in the > 2.00 mm class remained largely unchanged, indicating that in tall fescue, only specific diameter classes respond meaningfully to PGPR and moderate PL applications. These findings underscore the synergistic effects of PGPR and PL in enhancing root traits, particularly at moderate application levels, while also highlighting species-specific and diameter-class-specific responses.

Table 6 Effect of PGPR and PL litter on root length, surface area, and volume of annual ryegrass and tall fescue across root diameter classes

PL	PGPR	0–0.25 mm	0.26–0.50 mm	0.51–2.00 mm	> 2 mm
Annual ryegrass					
Run 1					
Length (cm)					
0% PL	No PGPR	10579.300a	1768.594c	1184.509c	33.835c
0% PL	PGPR	10838.502a	1848.208bc	1279.784bc	41.070bc
50% PL	No PGPR	11068.701a	1856.254abc	1339.387bc	44.251bc
50% PL	PGPR	11579.974a	2048.683abc	1557.553ab	67.623ab
75% PL	No PGPR	11244.208a	1949.932abc	1535.369ab	52.898abc
75% PL	PGPR	12457.183a	2152.134a	1736.698a	75.825a
100% PL	No PGPR	11079.901a	1842.674bc	1426.532abc	56.651abc
100% PL	PGPR	12359.159a	2085.694ab	1573.482ab	57.760abc
Surface area (cm^2)					
0% PL	No PGPR	352.598a	201.428c	283.493c	29.915c
0% PL	PGPR	358.477a	210.812abc	311.059bc	35.802c
50% PL	No PGPR	367.477a	211.467abc	326.533bc	39.408bc
50% PL	PGPR	390.039a	234.263abc	381.870ab	63.729ab
75% PL	No PGPR	377.771a	223.021abc	373.741ab	47.301abc
75% PL	PGPR	417.453a	244.268a	431.104a	69.060a
100% PL	No PGPR	376.545a	208.878bc	351.677abc	53.705abc
100% PL	PGPR	418.421a	238.186ab	381.203ab	54.964abc
Volume (cm^3)					
0% PL	No PGPR	29.915c	1.900c	6.157c	2.314d
0% PL	PGPR	35.802c	1.991abc	6.887bc	2.714 cd
50% PL	No PGPR	39.408bc	1.995abc	7.257bc	3.072bcd
50% PL	PGPR	63.729ab	2.219abc	8.573ab	5.414ab
75% PL	No PGPR	47.301ab	2.113abc	8.291ab	3.725abcd
75% PL	PGPR	69.060a	2.297a	9.786a	5.647a
100% PL	No PGPR	53.705abc	1.961bc	7.924abc	4.909abc
100% PL	PGPR	54.964ab	2.254ab	8.424ab	4.790abcd
Run 2					
Length (cm)					
0% PL	No PGPR	13263.005c	2509.955d	1217.428c	30.935c
0% PL	PGPR	14531.167bc	2651.080 cd	1522.203bc	46.608bc
50% PL	No PGPR	15077.195abc	2897.927bc	1660.777abc	64.237abc
50% PL	PGPR	17395.853a	3184.522ab	2119.160a	101.097a
75% PL	No PGPR	15805.052abc	3063.305ab	2003.695a	96.118a
75% PL	PGPR	17294.707a	3212.935a	2000.082ab	78.683ab
100% PL	No PGPR	16407.712ab	3104.602ab	1850.735ab	69.808abc
100% PL	PGPR	15619.762abc	2933.688abc	2045.802a	107.440a
Surface area (cm^2)					
0% PL	No PGPR	431.548c	287.022d	288.767c	27.403b
0% PL	PGPR	474.723bc	304.422 cd	366.903bc	41.980b
50% PL	No PGPR	497.335abc	331.482bc	405.213abc	61.588ab
50% PL	PGPR	568.452a	361.855ab	530.025a	98.123a
75% PL	No PGPR	519.410abc	353.160ab	494.868ab	94.562a
75% PL	PGPR	567.963a	367.847a	489.377ab	75.085ab
100% PL	No PGPR	541.157ab	355.942ab	452.328ab	64.827ab
100% PL	PGPR	512.248abc	335.503abc	512.288a	105.245a
Volume (cm^3)					
0% PL	No PGPR	27.403b	2.710d	6.217c	2.105b
0% PL	PGPR	41.980b	2.890 cd	8.047bc	3.335b
50% PL	No PGPR	61.588ab	3.133bc	9.012abc	5.453ab

Table 6 (continued)

PL	PGPR	0–0.25 mm	0.26–0.50 mm	0.51–2.00 mm	> 2 mm
50% PL	PGPR	98.123a	3.400ab	12.117a	9.015a
75% PL	No PGPR	94.562a	3.365ab	11.203ab	8.533a
75% PL	PGPR	75.085ab	3.485a	10.928ab	6.647ab
100% PL	No PGPR	64.827ab	3.373ab	10.097ab	5.450ab
100% PL	PGPR	105.245a	3.173abc	11.743a	9.612a
Tall fescue					
Run 1					
Length (cm)					
0% PL	No PGPR	5556.287a	488.105b	427.200a	3.198b
0% PL	PGPR	3854.903a	545.788ab	472.985a	4.878ab
50% PL	No PGPR	3856.780a	574.153ab	484.853a	4.465ab
50% PL	PGPR	4409.748a	619.675a	515.060a	7.683a
75% PL	No PGPR	3743.457a	567.228ab	480.803a	5.780ab
75% PL	PGPR	4478.620a	644.917a	500.170a	5.373ab
100% PL	No PGPR	3411.163a	534.470ab	431.752a	4.183ab
100% PL	PGPR	3990.872a	594.017ab	511.272a	4.405ab
Surface area (cm^2)					
0% PL	No PGPR	116.827b	55.358b	96.948a	2.673b
0% PL	PGPR	132.195ab	61.702ab	108.538a	4.092ab
50% PL	No PGPR	136.738ab	64.713ab	110.992a	3.858ab
50% PL	PGPR	152.497ab	70.102a	118.668a	6.723a
75% PL	No PGPR	131.263ab	64.317ab	112.848a	5.060ab
75% PL	PGPR	154.835a	72.715a	115.663a	4.410ab
100% PL	No PGPR	120.142ab	61.197ab	98.108a	3.760ab
100% PL	PGPR	138.113ab	67.085ab	118.395a	3.707ab
Volume (cm^3)					
0% PL	No PGPR	2.673b	0.522b	1.920	0.188b
0% PL	PGPR	4.092ab	0.578ab	2.185	0.292ab
50% PL	No PGPR	3.858ab	0.603ab	2.237	0.285ab
50% PL	PGPR	6.723a	0.657a	2.410	0.513a
75% PL	No PGPR	5.060ab	0.605ab	2.352	0.392ab
75% PL	PGPR	4.410ab	0.680a	2.367	0.302ab
100% PL	No PGPR	3.760ab	0.582ab	1.972	0.303ab
100% PL	PGPR	3.707ab	0.627ab	2.405	0.275ab
Run 2					
Length (cm)					
0% PL	No PGPR	2456.595c	394.852d	122.547e	3.543a
0% PL	PGPR	3361.218b	511.672c	178.447de	2.892a
50% PL	No PGPR	3381.645b	583.213bc	250.66 cd	4.450a
50% PL	PGPR	4097.53ab	639.093ab	325.55abc	3.853a
75% PL	No PGPR	3593.375b	581.463bc	279.273bc	3.538a
75% PL	PGPR	4433.693a	677.795ab	387.288a	3.805a
100% PL	No PGPR	3928.467ab	617.172abc	354.483ab	4.148a
100% PL	PGPR	4621.803a	692.505a	408.848a	4.097a
Surface area (cm^2)					
0% PL	No PGPR	82.422c	43.675d	29.293e	2.707a
0% PL	PGPR	113.232b	57.318c	41.253de	2.255a
50% PL	No PGPR	111.495b	66.925bc	56.558 cd	3.723a
50% PL	PGPR	136.263ab	73.043ab	72.702abc	3.293a
75% PL	No PGPR	120.208b	66.373bc	62.925bc	3.123a
75% PL	PGPR	149.515a	78.565ab	83.093a	3.375a
100% PL	No PGPR	131.105ab	70.905ab	78.452ab	3.590a
100% PL	PGPR	157.007a	79.903a	89.810a	3.475a

Table 6 (continued)

PL	PGPR	0–0.25 mm	0.26–0.50 mm	0.51–2.00 mm	> 2 mm
Volume (cm^3)					
0% PL	No PGPR	2.707a	0.398e	0.637e	0.172a
0% PL	PGPR	2.255a	0.53d	0.862d	0.148a
50% PL	No PGPR	3.723a	0.637bcd	1.145 cd	0.26a
50% PL	PGPR	3.293a	0.69abc	1.433abc	0.24a
75% PL	No PGPR	3.123a	0.627 cd	1.257bc	0.237a
75% PL	PGPR	3.375a	0.752ab	1.558ab	0.253a
100% PL	No PGPR	3.59a	0.672abc	1.525abc	0.265a
100% PL	PGPR	3.475a	0.763a	1.737a	0.255a

Root traits are classified by diameter classes (0–0.25 mm, 0.26–0.50 mm, 0.51–2.00 mm, > 2 mm). Different letters within a column indicate significant differences at $p < 0.05$

4 Discussion

In this study, seedling emergence of annual ryegrass and tall fescue was not significantly affected by the bacillus-based PGPR applied. This finding aligns with previous research indicating that under non-stress conditions, PGPR may not substantially enhance germination [58–60]. However, several studies have reported that PGPR can improve seed germination under abiotic stress conditions. For instance, improved germination has been observed in wheat (*Triticum aestivum*) under drought [58] and cadmium stresses [59], and in chickpea (*Cicer arietinum*) [60], wheat [61], mustard (*Brassica juncea*) [62], sea asparagus (*Salicornia ramosissima*) [63], lettuce (*Lactuca sativa*), and radish (*Raphanus sativus*) [64] under salinity stress following PGPR inoculation. Enhanced germination of *S. ramosissima* under high temperature conditions has also been reported following PGPR inoculation [63]. These improvements under stress conditions are largely attributed to the hormonal activity of PGPR, including the production of gibberellic acid, indole-3-acetic acid (IAA), cytokinins, and ethylene, which regulate cell division, and stress adaptation mechanisms [16, 65].

Inoculation of annual ryegrass and tall fescue with PGPR has been shown to promote shoot height and tillering at different PL rates. Annual ryegrass exhibited the greatest response to PL and PGPR. In both species, PGPR treatments at reduced PL levels exhibited plant heights and tiller numbers comparable to or greater than those observed at 100% PL without PGPR. The shortest plants and lowest number of tillers consistently occurred in the 0PL treatment without PGPR, while PGPR alone slightly improved growth. These findings suggest that PGPR can partially substitute for high PL fertilization, supporting more sustainable forage production. The association between plant roots and beneficial rhizobacteria represents a complex and highly effective interaction that supports plant growth and development [9]. A key feature of this relationship is the production of phytohormones by PGPR, which plays a critical role in promoting vegetative growth. In addition to hormonal regulation, PGPR contribute to essential processes such as atmospheric nitrogen fixation, solubilization of phosphorus and potassium, and siderophore production, thereby enhancing nutrient availability [65]. Collectively, these mechanisms act synergistically to improve the overall growth performance including the height and number of tillers of annual ryegrass and tall fescue. Wanget al. [66] reported that reduction of NPK fertilization by 25% with inoculation of PGPR had similar height and tillers compared to full fertilization in wheat (*Triticum aestivum*). Errricksonet al. [67] reported a higher tiller production rate in creeping bentgrass (*Agrostis stolonifera*) following the inoculation of *Paraburkholderia aspalathi*. Similarly, Ker [68] observed

that inoculation of switchgrass (*Panicum virgatum*) with PGPR resulted in great tiller production and stand establishment. This may be associated with the ability of the Bacillus PGPR consortium to interfere with key hormones including 1-aminocyclopropane-1-carboxylic acid (ACC) and ethylene synthesis which play an important role in cell division. According to Brown [69] the metabolites synthesized by PGPR such as auxins, gibberellins, cytokinin, and their precursors, play a vital role in enhancing plant development. These bioactive molecules are involved in regulating various physiological processes that collectively contribute to plant growth [37]. As noted by Taizet al. [70], Egamberdievaet al. [71], Akhtaret al. [72] and Shahet al. [73] auxins, cytokinin and gibberellins are known to stimulate the elongation of shoots, roots, and leaves by promoting cell division, expansion, and stretching within meristematic zones.

The positive effects of PGPR at low application rates extended to both shoot and root dry biomass in annual ryegrass and tall fescue, particularly at moderate PL levels (50% PL and 75% PL). PGPR also enhanced biomass under low-fertility conditions (0PL), demonstrating its effectiveness even without PL, however, 0% PL did not match the full PL rate for biomass production indicating that at very low nutrient levels (0% PL), nutrient limitations may be too severe for PGPR to compensate. At moderate PL rates, shoot and root biomass with PGPR often equaled or in some cases exceeded that observed under 100% PL without PGPR, highlighting the potential of PGPR to maintain or improve forage grass growth and yield with reduced fertilizer input. At a full (100%) PL rate, the effectiveness of PGPR was reduced, suggesting that high nutrient concentrations may suppress microbial activity and diminish PGPR inoculation efficiency [6, 73]. This aligns with findings by Bhattaraiet al. [74] who observed that PGPR perform more effectively when inoculated into low fertile soil. This phenomenon has also been reported in several studies, where legume–rhizobia symbiosis showed reduced biological nitrogen fixation (BNF) efficiency in nitrogen-rich soils, emphasizing that resource scarcity or environmental stress can increase plant dependence on PGPR for enhanced functionality [75, 76]. The increase in annual ryegrass and tall fescue yields under reduced mineral fertilizer conditions with PGPR application can be attributed to a combination of enhanced nutrient released from PL, phosphate and potassium solubilisation, siderophore production, nitrogen fixation, stimulation of phytohormone production and the activation of metabolic pathways that promote plant growth and development [9, 77–80]. Yolcuet al. [81] reported increased shoot dry biomass in Italian ryegrass when PGPR was applied alongside manure, compared to manure alone. Similarly, Sullins [38] found that bermudagrass forage yield with PGPR combined with half the nitrogen rate was comparable to yields from full N fertilizer application, suggesting that PGPR can sustain forage productivity in hay and grazing systems while reducing fertilizer input.

In both species, PGPR significantly improved root system development when combined with moderate PL rates (50–75% PL). Root length, surface area, volume, and branching traits were consistently higher under 50% PL + PGPR and 75% PL + PGPR compared to 100% PL without PGPR, often by 10–30% or more, highlighting the advantage of integrated nutrient and microbial management over heavy fertilization alone. This effect may be attributed to the ability of PGPR to modulate key hormonal signaling pathways involved in root development. Root system architecture is frequently influenced by PGPR interactions with hormone-regulated pathways, particularly those involving auxin, cytokinin, ethylene, and, to a lesser extent, gibberellin and abscisic acid

[82–85]. The auxin-to-cytokinin ratio is especially critical for regulating organogenesis and root patterning, and PGPR can influence this balance by producing phytohormones (e.g., auxins and cytokinins) and secondary metabolites that modulate hormone signaling [86]. When root traits of annual ryegrass and tall fescue were analyzed by diameter class, PGPR inoculation notably enhanced root development in the 0.51–2.00 mm and >2 mm classes, particularly when combined with moderate poultry litter rates (50–75PL). In both species, treatments with 50% PL + PGPR and 75% PL + PGPR consistently equaled or exceeded root development observed under 100% PL without PGPR. These results underscore the potential of integrating biological inoculants with moderate nutrient inputs to optimize root architecture and improve resource acquisition efficiency. Several *Bacillus* spp. strains have been reported to enhance root weight, length, diameter, surface area, and volume in various plant species [87–89]. The impact of PGPR on crop yields has been widely studied, yet questions remain regarding their practical application in field conditions. While PGPR cannot entirely replace synthetic fertilizers, they offer potential to reduce agrochemical use in agriculture [73]. This study demonstrates the practical use of bacillus-based PGPR consortia as biofertilizers in forage production systems, with a focus on agronomic performance under greenhouse conditions. Notable differences were observed, with the application of 50–75% PL combined with PGPR producing growth and yield outcomes comparable to those under full PL fertilization for both annual ryegrass and tall fescue. However, responses were more consistent in annual ryegrass, suggesting a possible role of host specificity in plant–microbe interactions. Additionally, PGPR combined with moderate PL rates significantly enhanced root system development, leading to improved water and nutrient uptake and ultimately increased shoot biomass.

5 Conclusion

In annual ryegrass and tall fescue forage systems, the application of PGPR presents a promising strategy for reducing PL inputs. This study demonstrates that PL rates can be reduced by 25–50% with PGPR without significantly compromising the yield. Moreover, combined application of moderate PL rate with PGPR, increased root system development in the two species, supporting better nutrient and water uptake. The combined benefits of lower input costs, improved environmental sustainability, and yields comparable to those achieved with 100% PL make this approach especially appealing for forage production. Although the study was conducted in a greenhouse, the results provide a strong foundation for the use of PGPR in forage systems. Future field experiments will be a critical step toward full-scale implementation.

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Author contributions
All authors contributed equally.

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Data availability
The data used in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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