Effect of Plant Growth-Promoting Rhizobacteria at Various Nitrogen Rates on Corn Growth

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Abstract

Plant growth-promoting rhizobacteria (PGPR) colonize plant roots and promote plant growth by producing and secreting various chemical regulators in the rhizosphere. With the recent interest in sustainable agriculture, an increasing number of researchers are investigating ways to improve the efficiency of PGPR use to reduce chemical fertilizer inputs needed for crop production. Accordingly, greenhouse studies were conducted to evaluate the impact of PGPR inoculants on biomass production and nitrogen (N) content of corn (Zea mays L.) under different N levels. Treatments included three PGPR inoculants (two mixtures of PGPR strains and one control without PGPR) and five N application levels (0%, 25%, 50%, 75%, and 100% of the recommended N rate of 135 kg N ha⁻¹). Results showed that inoculation of PGPR significantly increased plant height, stem diameter, leaf area, and root morphology of corn compared to no PGPR application under the same N levels at the V6 growth stage, but few differences were observed at the V4 stage. PGPR with 50% of the full N rate produced corn biomass and N concentrations equivalent to or greater than that of the full N rate without inoculants at the VT stage. In conclusion, mixtures of PGPR can potentially reduce inorganic N fertilization without affecting corn plant growth parameters. Future research is needed under field conditions to determine if these PGPR inoculants can be integrated as a bio-fertilizer in crop production nutrient management strategies.

Keywords

Plant Growth-Promoting Rhizobacteria, Corn Growth, Nitrogen Fertilization, Root Morphology, Nitrogen Use Efficiency
1. Introduction

Commercial fertilizers, especially N sources, are essential for maintaining global crop production and fulfilling food requirements for a rapidly growing world population with limited land resources [1] [2] [3] [4]. In 2014, over 11.7 million tonnes of N fertilizer were applied to US agricultural crops [5]. This number is expected to increase in the coming years because inorganic N is an indispensable input in crop production. For example, Stewart et al. [1] evaluated several long-term studies to determine the effect of eliminating N fertilizer, and predicted that corn and cotton (Gossypium hirsutum L.) yields would decline by 41% and 37%, respectively, without N fertilizer. Optimal crop yields also depend upon the nitrogen use efficiency (NUE) of crops. Generally, NUE is very low (~33%; [6]) due to various soil processes and environmental factors [7] [8]. For example, over half of the N applied can be lost from agricultural systems as gaseous loss (N2, nitrous oxide, NH3 etc.), runoff (NO3), or leaching (NO3) into groundwater [9] [10]. Changing this poor NUE requires more effective management practices.

Microorganisms that promote plant growth may be worth evaluating as a prospective tool to improve fertilizer use efficiency [11] [12] [13] [14] [15]. Plant growth-promoting rhizobacteria (PGPR) are free-living microbes that live on or around the roots [16] and that stimulate plant growth and enhance root development and architecture [17] [18] [19] [20]. Kumar et al. [21] reported that applying Pseudomonas aeruginosa LES4 at half the recommended fertilizer rate resulted in growth of sesame (Sesamum indicum L.) that was equivalent to treatments at the full fertilizer rate, and that the oil yield increased 33.3%, and protein yield increased 47.5% compared to the full fertilizer rate. Adesemoye et al. [19] found that on tomato (Solanum lycopersicum) supplementing 75% of the recommended fertilizer with a mixture of Bacillus spp. and arbuscular mycorrhiza fungus (AMF) resulted in growth, yield, and uptake of N and P equivalent to the full fertilizer rate without inoculants. Similar results also showed that inoculating P. thivervalensis and Serratia marcesens to soil with 75% of the recommended chemical fertilizer rate for corn [22] and inoculating Rhodopseudomonas palustris to soil with 50% of recommended chemical fertilizer rate for Chinese cabbage (Brassica rapachinesis; [23]) resulted in the same plant biomass and yield as with the full rate.

Among the genera of PGPR, Bacillus is the most widely used to enhance plant growth and suppress plant diseases [24] due to their capacity to form stable endospores that can be inoculated onto crop seeds. Also, their wide metabolic capabilities allows them to play important roles in soil ecosystem functions and processes, such as soil carbon, nitrogen, and sulfur cycling, and transformation of other soil nutrients [25]. Huang et al. [26] isolated four Bacillus strains from rainforest soils that increased plant height and shoot biomass of Arabidopsis, corn, and tomato under greenhouse conditions. In another study, Wani and Khan [27] reported that Bacillus strains enhanced plant height and plant fresh
weigh of tomatoes in both nutrient poor soils and soils receiving N fertilization. However, the range of enhancement was much lower when sufficient N was supplied. Inoculating plants with *Bacillus* strain PSB10 also resulted in enhanced nodulation, chlorophyll, leghemoglobin, seed yield, and grain protein of chickpea (*Cicer arietinum* L.) in chromium-stressed soils [27]. In another study, Meng et al. [28] inoculated nine types of plants under greenhouse conditions with the *B. velezensis* strain and found that some of the plants increased growth at various levels in different plant parts. Growth promotion by *Bacillus* has also been observed with canola (*Brassica napus* L., [29]), corn [30], soybean (*Glycine max*, [31]), sugar beet (*Beta vulgaris* [32]), and wheat (*Triticum aestivum* L., [33]).

Numerous studies and reviews have reported plant growth promotion, increased yield, phytohormone production, soil P solubilization, and enhanced N uptake through inoculation with *Bacillus* spp. However, most of these studies were conducted using single-strain inoculations and the positive effects were only shown under specific conditions, and hence, growth promotion was limited when using single-strain inoculations [34]. For example, *B. velezensis* inoculation increased dry leaf weight, but not root weight for several vegetative crops [28]. In a study on canola, de Freitas et al. [29] reported that *Bacillus* spp. had no effect on plant growth when rock phosphate was applied; while seed yield was increased, there was no effect on P uptake with triple superphosphate. Similarly, de Freitas [33] reported in a pot study that *B. polymyxa* tended to enhance wheat grain yield, but no differences in total-N or shoot dry matter yield were observed as compared to the uninoculated control.

A few studies have reported that mixtures of PGPR strains generally cause more consistent positive effects on plant growth than do individual strains [35] [36] [37]. In addition, some studies have suggested that PGPR are more effective under limited nutrient conditions [26] [38] [39]. For example, a greenhouse study showed that *B. polymyxa* had a better stimulatory effect on corn plant growth and N, P, and K uptake in nutrient-deficient soils than in nutrient-rich soils [39]. However, limited information exists concerning the effects of *Bacillus* spp. mixtures on corn growth with reduced levels of N fertilizers. Therefore, the objectives of this study were to: 1) evaluate the impact of PGPR mixtures on corn root growth and biomass production under different N levels; 2) investigate the potential of PGPR mixtures to allow a reduction in the amount of inorganic N fertilizer needed by resulting in corn plant growth and nutrient uptake levels equivalent to those at the recommended N fertilizer rate; and 3) determine the optimal N rate for stimulating PGPR growth-promoting effects on corn.

### 2. Materials and Methods

#### 2.1. Greenhouse Experiment

A greenhouse container study was conducted at Auburn University’s Horticulture Paterson Greenhouse (HP) in Auburn, AL, USA. This study consisted of two separate experiments conducted with the same treatments. The first experi-
ment was conducted from March to May and second experiment from April to June of 2017 in the same greenhouse. The soil used for this study was a Kalmia sandy loam (fine-loamy over sandy, siliceous, semiactive, thermic Typic Hapludults) collected from the E.V. Smith Research Center-Plant Breeding Unit in Elmore County, near Tallassee, AL. Surface soil (0 - 15 cm depth) was collected in early-spring from an area that had been previously under row crop production. The soil was sieved through a 5-mm sieve and was analyzed for nutrient concentrations according to procedures described by Hue and Evans [40]. Briefly, the soil had a pH of 5.5, total N concentration of 0.5 g∙kg⁻¹, total C concentration of 4.8 g∙kg⁻¹, P concentration of 22.7 mg∙kg⁻¹, K concentration of 58.1 mg∙kg⁻¹, Ca concentration of 199 mg∙kg⁻¹, and Mg concentration of 51.5 mg∙kg⁻¹. Based on initial soil pH and nutrient levels, the Alabama Agricultural Extension System recommended applying 45 kg P ha⁻¹, 45 kg K ha⁻¹, and 4.5 tons ha⁻¹ limestone for corn production.

The experiment was conducted as a completely randomized design with five replications. Treatments consisted of three PGPR inoculants combined with five N rates. The PGPR treatments consisted of two PGPR strain mixtures (Table 1) and one control without PGPR. The strains were obtained from pure culture collections at Auburn University’s Department of Entomology and Plant Pathology. These strains have positive effects on plant growth and were selected from previous screening experiments. The bacterial mixtures were prepared by mixing each strain’s spore, which was previously quantified by plating the suspension on tryptic soy agar (TSA) plates and incubating for 48 h at 25°C, in equal concentrations. A bacterial mixture of 1 × 10⁶ spore ml⁻¹ was used for this study. The N rate treatments consisted of applying 0%, 25%, 50%, 75%, and 100% of the 135 kg N ha⁻¹ rate recommended by Alabama Cooperative Extension System for corn on a Coastal Plain soil [41]. One day prior to sowing, urea (46% N), triple superphosphate, and potassium chloride dissolved in water were added to the soil.

The experimental units consisted of plastic containers (8 L Gro Pro square pots, Sunlight Supply, Inc., Vancouver, WA, USA) that were 24 cm tall, measured 23 × 23 cm at the top, and tapered to 18 × 18 cm at the base. The containers

| PGPR Mix # | Original Strain # | Identification          |
|-----------|-------------------|-------------------------|
| 1         | 2RA-17            | *Bacillus cereus*       |
|           | 99-101            | *B. amyloliquefaciens*  |
|           | 33B-9             | *B. mojavensis*         |
|           | IN-937a           | *B. subtilis subsp. subtilis* |
| 2         | SE-52             | *B. safensis*           |
|           | INR-7             | *B. altitudinis*        |
|           | SE-56             | *Lysinibacillus xylanilyticus* |
|           | E-681             | *Paenibacillus peoriae* |
were filled with 12.5 kg of soil and adjusted to saturation with water. Five extra containers were designated for determining saturation. Saturation was estimated by determining the average amount of water needed to fill containers until they reached a drip point (i.e., when water begins to drip from basal drain holes). Two corn seeds (P1319HR; DuPont Pioneer, Johnston, IA, USA) per container were sown in moist soil to a depth of 5 cm. A 1 ml suspension of the respective bacterial mixture (*Bacillus* spp.) was applied on top of each seed at sowing. After germination, plants were thinned to one plant per container and watered every three days to saturation. Temperature within the greenhouse was maintained at 26˚C ± 2˚C during the day and 20˚C ± 3˚C at night. To minimize micro-environmental variation among treatments, containers were rotated weekly at random by treatment.

### 2.2. Data Collection

Corn plants were harvested at the V4, V6, and VT vegetative growth stages. Plant height, stem diameter, leaf area, leaf chlorophyll content, root morphological features, and dry shoot and root weights were measured at each harvest time. Plant height was determined by measuring from the base to the top of the newest fully developed leaf. Stem diameter was determined at the base of plant using high-precision digital calipers (MitutoyoDigimatic Caliper, Mitutoyo Corp., Kawasaki, Japan). Leaf greenness (chlorophyll content) was determined by measuring from the newest fully expanded functional corn leaf with a Minolta SPAD 502 plus (Minolta Camera Co., Ltd., Osaka, Japan). Afterwards, plants were cut at the soil surface with handheld pruning shears. Leaf area was determined from the harvested plants using an area meter (LI-3100C Area Meter, LI-COR Biosciences, Lincoln, NE, USA). All leaves from one plant were cut and placed on an area meter one by one (avoiding overlap) to determine leaf area. Root biomass was determined by carefully rinsing roots on a 0.5 mm mesh screen sieve. The above- or below-ground plant biomass was then placed into paper bags and dried (55˚C) until the weight became constant in a forced-air drying oven to determine dry weight. Before drying, roots were scanned and analyzed for root morphology using the WinRHIZO Arabidopsis software (v2009c 32 bit system, Regent Instruments, Quebec, QC, Canada) connected to an Epson XL 10,000 professional scanner (Seike Epson Corp., Shinjuku, Tokyo, Japan). Each individual root system was evenly spread apart, placed in a water bath on a transparent tray (30 × 40 cm width), and imaged at a resolution of 157.5 dots per cm as described by Bauhus and Messier [42] and Costa et al. [43]. The following root characteristics were determined: total root length (cm), root surface area (cm²), root volume (cm³), and average root diameter (mm). Plant total N was determined on the dried shoot and root tissues. Ground plant tissues (0.2 mm mesh) of leaves, stems, and roots harvested at the VT stage were analyzed for N using the dry combustion method (LECO FP-528 Nitrogen/Protein Analyzer, LECO Corp., Saint Joseph, MI, USA).
2.3. Data Analysis

An analysis of variance (ANOVA), using a general linear model (GLM) of SAS 9.4 [44], was used to analyze each response variable in this experiment. The least significant difference test (LSD) at a 0.05 probability level was used to identify significant differences among treatments. Significant interactions ($P \leq 0.05$) were observed between the two experiments and the N rates. Thus, treatment means for each N rate were analyzed separately by experiment. Significant interactions between N rate and PGPR treatments ($P \leq 0.05$) were observed for some response variables, thus, the LSD test was conducted to identify significant differences among PGPR treatments at each N level for these response variables. Also, comparisons were made to determine the effects of each PGPR inoculant at each N level to the non-inoculated full N rate treatment (standard application rate) using the LSD test.

3. Results and Discussion

3.1. Plant Growth Parameters

Plant height is often correlated with the number of leaves per plant and can potentially affect corn yield [45]. Nitrogen levels significantly affected the corn vegetative growth parameters evaluated (plant height, stem diameter, and leaf area) in this study from the V4 to VT stages (Table 2 and Table 3). There were no significant differences in either experiment or clear tendencies observed among the N levels evaluated for plant height at the V4 and VT stages in either experiment. Plants receiving 75% ($P = 0.0052$) and 100% ($P = 0.0327$) of the recommended N rate were significantly taller than those with no N application at the V6 stage in the first experiment (HP1). Our results were consistent with previous studies, which showed that the tallest plants were observed with the application of approximately 70% of recommended N rate [46] [47]. Arnon [48] indicated that shorter plants resulting from low N availability may be associated with delayed cell division at the growing points. In addition to nutrient content of soil, plant height is also influenced by soil moisture, temperature, sunlight duration, and other environmental factors. Soil moisture and temperature were suitable for plant growth under the greenhouse conditions of this study, and thus, all plants had normal plant height irregardless of N levels. Significant effects of microbial inoculations (averaged across N rates) on plant height were only observed at the V6 stage in HP1 (Table 2), in which, PGPR strain mixture 1 increased plant height on average by 6.8% and 11.0% compared to the no-PGPR ($P = 0.0534$) and PGPR strain mixture 2 ($P = 0.0073$), respectively. Although there were no statistical differences between PGPR inoculants and non-inoculated treatments at the V4 and VT stages for either experiments (Table 2 and Table 3), PGPR inoculations tended to increase plant height during these growth stages. For example, the tallest plant was observed for PGPR mixture 1 when combined with 25% of recommended N rate (N25P1) at the V4 stage, 50% of
Table 2. PGPR effects on corn plant height, stem diameter, leaf area, and SPAD reading as influenced by N rates during the V4, V6, and VT growth stages in the Horticulture Paterson Greenhouse from March to May (HI).

| Treatment† | Plant height (cm) | Stem diameter (mm) | Leaf area (cm²) | SPAD readings |
|------------|-------------------|--------------------|-----------------|---------------|
|            | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    |
| N0P0       | 42.2 ± 1.96 | 53.4 ± 4.55 | 166.2 ± 4.94 | 6.54 ± 0.36 | 15.76 ± 0.86 | 14.0 ± 0.61 | 200.2 ± 27.5 | 1126 ± 114 | 1932 ± 107 | 38.9 ± 0.86 | 39.2 ± 1.39 | 19.8 ± 0.76 |
| N0P1       | 43.2 ± 3.76 | 61.8 ± 1.03 | 173.3 ± 6.84 | 6.99 ± 0.22 | 14.95 ± 1.43 | 13.0 ± 0.73 | 205.0 ± 9.54 | 1280 ± 53.8 | 1904 ± 102 | 42.0 ± 1.50 | 42.8 ± 0.31 | 21.0 ± 0.77 |
| N0P2       | 49.8 ± 0.75 | 49.3 ± 1.49 | 170.2 ± 13.8 | 6.15 ± 0.38 | 13.21 ± 0.85 | 13.6 ± 0.70 | 205.2 ± 7.53 | 1012 ± 64.4 | 1943 ± 136 | 39.3 ± 1.79 | 38.3 ± 2.02 | 23.9 ± 1.10 |
| N25P0      | 40.0 ± 4.71 | 62.5 ± 1.32 | 176.6 ± 13.3 | 3.53 ± 0.34 | 17.07 ± 0.61 | 14.5 ± 0.58 | 139.6 ± 23.4 | 1255 ± 31.2 | 1936 ± 81.2 | 41.8 ± 2.05 | 38.7 ± 1.25 | 23.0 ± 0.52 |
| N25P1      | 44.6 ± 4.23 | 62.0 ± 4.36 | 165.6 ± 10.8 | 5.11 ± 0.74 | 16.04 ± 0.83 | 14.4 ± 0.41 | 195.2 ± 26.3 | 1270 ± 95.5 | 2117 ± 48.8 | 40.1 ± 1.37 | 39.3 ± 1.25 | 23.5 ± 0.22 |
| N25P2      | 44.6 ± 1.78 | 55.0 ± 3.21 | 181.3 ± 7.55 | 6.68 ± 0.48 | 15.92 ± 0.50 | 15.3 ± 0.81 | 210.7 ± 26.9 | 1051 ± 91.9 | 1987 ± 53.0 | 40.8 ± 1.60 | 41.4 ± 0.85 | 27.5 ± 1.30 |
| N50P0      | 45.3 ± 2.63 | 54.6 ± 2.52 | 179.3 ± 12.0 | 4.59 ± 0.20 | 15.44 ± 0.62 | 15.7 ± 0.93 | 217.4 ± 18.1 | 1196 ± 122 | 2021 ± 138 | 40.3 ± 2.02 | 38.3 ± 0.99 | 26.9 ± 0.44 |
| N50P1      | 39.6 ± 2.42 | 66.0 ± 3.24 | 180.0 ± 12.6 | 5.23 ± 1.03 | 14.25 ± 0.81 | 15.1 ± 0.75 | 193.5 ± 30.8 | 1349 ± 78.9 | 1689 ± 109 | 40.5 ± 0.47 | 43.1 ± 0.71 | 25.7 ± 1.23 |
| N50P2      | 40.5 ± 3.97 | 61.0 ± 2.17 | 172.4 ± 6.90 | 4.33 ± 0.45 | 16.44 ± 0.88 | 16.0 ± 0.51 | 168.4 ± 12.7 | 1385 ± 100 | 1817 ± 135 | 39.5 ± 1.77 | 44.3 ± 1.00 | 27.8 ± 0.84 |
| N75P0      | 39.5 ± 5.76 | 66.0 ± 2.00 | 183.8 ± 4.59 | 4.61 ± 0.39 | 16.85 ± 0.51 | 15.8 ± 0.43 | 188.5 ± 31.3 | 1377 ± 85.4 | 1777 ± 121 | 42.7 ± 1.98 | 44.2 ± 0.86 | 27.1 ± 1.26 |
| N75P1      | 44.8 ± 3.43 | 65.3 ± 1.33 | 166.6 ± 7.56 | 4.48 ± 0.60 | 13.61 ± 0.19 | 15.3 ± 0.54 | 181.3 ± 15.4 | 1233 ± 33.0 | 1882 ± 155 | 41.3 ± 1.41 | 41.7 ± 1.07 | 27.5 ± 0.98 |
| N75P2      | 45.2 ± 1.71 | 61.0 ± 5.40 | 179.0 ± 13.3 | 5.70 ± 0.62 | 13.78 ± 0.90 | 15.7 ± 0.60 | 183.1 ± 29.5 | 1081 ± 93.7 | 1854 ± 107 | 40.8 ± 1.56 | 41.1 ± 0.93 | 30.9 ± 2.29 |
| N100P0     | 38.6 ± 2.82 | 61.6 ± 1.33 | 162.5 ± 7.33 | 5.32 ± 0.84 | 15.06 ± 0.55 | 16.5 ± 0.89 | 188.9 ± 40.3 | 1382 ± 91.1 | 1867 ± 133 | 40.0 ± 1.42 | 42.7 ± 1.28 | 28.2 ± 1.58 |
| N100P1     | 40.3 ± 3.68 | 63.4 ± 3.36 | 177.8 ± 8.35 | 5.60 ± 0.93 | 14.96 ± 0.59 | 15.6 ± 0.57 | 181.9 ± 23.1 | 1460 ± 83.1 | 1943 ± 109 | 43.9 ± 3.34 | 45.4 ± 0.85 | 29.1 ± 0.71 |
| N100P2     | 42.8 ± 3.09 | 60.8 ± 2.69 | 170.0 ± 3.11 | 5.23 ± 0.33 | 14.15 ± 0.46 | 16.0 ± 0.86 | 169.7 ± 14.9 | 1432 ± 123 | 1950 ± 70.1 | 42.2 ± 2.51 | 43.8 ± 0.95 | 30.2 ± 0.62 |

P > F (0.05)

| N          | 0.5452 | 0.0087 | 0.8165 | 0.0044 | 0.0508 | 0.0002 | 0.7751 | 0.0064 | 0.3105 | 0.5512 | 0.0002 | <0.0001 |
| PGPR       | 0.2493 | 0.0092 | 0.9451 | 0.1733 | 0.0886 | 0.2407 | 0.9538 | 0.1106 | 0.9982 | 0.5992 | 0.0457 | <0.0001 |
| N*PGPR     | 0.6657 | 0.2295 | 0.738  | 0.0983 | 0.0843 | 0.9932 | 0.6329 | 0.2265 | 0.6174 | 0.7733 | 0.0035 | 0.6256 |

†N—nitrogen fertilizer; P0—no PGPR; P1—PGPR mixture 1; P2—PGPR mixture 2; N0, N25, N50, N75, and N100—0%, 25%, 50%, 75%, and 100% of the recommended N rate, respectively. †Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at P < 0.05 among PGPR treatments under the same N rate.
Table 3. PGPR effects on corn plant height, stem diameter, leaf area, and SPAD reading as influenced by N rates during the V4, V6, and VT growth stages in the Horticulture Paterson Greenhouse from April to June (HP2).

| Treatment† | Plant height (cm) | Stem diameter (mm) | Leaf area (cm²) | SPAD readings |
|------------|-------------------|--------------------|----------------|---------------|
|            | V4 | V6 | VT | V4 | V6 | VT | V4 | V6 | VT | V4 | V6 | VT |
| N0P0       | 54.8 ± 3.37 | 58.8 ± 1.91 | 193.0 ± 9.17 | 9.01 ± 1.25 | 13.90 ± 0.94 | 16.9 ± 0.58 | 445.3 ± 96.6 | 905.5 ± 65.7 | 2530 ± 170 | 46.6 ± 1.10 | 42.9 ± 1.43 | 25.4 ± 1.72 |
| N0P1       | 49.2 ± 4.21 | 59.0 ± 3.79 | 196.0 ± 6.51 | 10.0 ± 0.97 | 13.91 ± 1.38 | 16.2 ± 0.60 | 448.2 ± 68.2 | 1158 ± 135 | 2644 ± 181 | 49.2 ± 1.35 | 48.0 ± 2.52 | 26.3 ± 0.86 |
| N0P2       | 53.5 ± 3.65 | 54.6 ± 3.46 | 179.8 ± 9.33 | 11.2 ± 0.98 | 14.92 ± 0.96 | 16.2 ± 0.84 | 464.4 ± 40.4 | 1060 ± 72.4 | 3246 ± 504 | 47.2 ± 1.84 | 46.2 ± 1.40 | 29.7 ± 2.73 |
| N25P0      | 44.4 ± 0.68 | 59.8 ± 4.50 | 184.4 ± 12.8 | 9.51 ± 0.43 | 14.85 ± 0.64 | 15.5 ± 0.80 | 441.1 ± 52.4 | 1001 ± 63.0 | 2645 ± 144 | 47.0 ± 1.76 | 43.7 ± 1.71 | 32.0 ± 2.13 |
| N25P1      | 55.8 ± 3.22 | 54.0 ± 3.13 | 182.8 ± 9.26 | 9.62 ± 0.81 | 14.10 ± 0.66 | 16.0 ± 0.74 | 455.0 ± 52.0 | 1016 ± 90.7 | 2605 ± 216 | 48.3 ± 1.21 | 44.0 ± 1.33 | 31.2 ± 0.97 |
| N25P2      | 49.8 ± 4.71 | 57.5 ± 4.33 | 188.6 ± 13.7 | 10.4 ± 0.50 | 15.08 ± 0.70 | 16.4 ± 0.88 | 536.4 ± 42.5 | 967.8 ± 21.3 | 2582 ± 162 | 48.2 ± 1.50 | 44.8 ± 0.49 | 30.9 ± 1.37 |
| N50P0      | 51.0 ± 4.28 | 54.0 ± 3.30 | 191.4 ± 4.43 | 9.72 ± 0.84 | 14.75 ± 0.44 | 17.4 ± 0.67 | 462.4 ± 39.3 | 1119 ± 80.2 | 2841 ± 220 | 47.8 ± 1.16 | 43.9 ± 0.64 | 37.3 ± 2.20 |
| N50P1      | 57.3 ± 2.50 | 52.0 ± 2.65 | 199.5 ± 12.1 | 9.80 ± 0.83 | 15.58 ± 0.23 | 17.0 ± 0.29 | 511.9 ± 70.4 | 970.5 ± 45.0 | 2640 ± 155 | 49.0 ± 1.40 | 45.7 ± 1.86 | 41.1 ± 1.78 |
| N50P2      | 55.2 ± 3.38 | 58.2 ± 4.19 | 170.8 ± 10.7 | 9.49 ± 0.94 | 16.07 ± 0.20 | 17.3 ± 0.40 | 455.2 ± 63.1 | 1122 ± 46.8 | 2858 ± 189 | 50.0 ± 1.33 | 45.2 ± 0.83 | 39.1 ± 0.96 |
| N75P0      | 48.3 ± 4.67 | 53.5 ± 2.40 | 168.5 ± 9.19 | 11.4 ± 0.37 | 14.59 ± 0.65 | 16.2 ± 0.47 | 561.5 ± 54.8 | 1017 ± 47.8 | 3159 ± 380 | 49.2 ± 0.64 | 43.7 ± 2.30 | 36.7 ± 3.45 |
| N75P1      | 48.8 ± 3.51 | 57.3 ± 2.46 | 189.0 ± 10.8 | 9.64 ± 0.70 | 15.62 ± 1.02 | 16.4 ± 0.66 | 429.6 ± 77.9 | 1024 ± 28.3 | 2831 ± 135 | 46.1 ± 1.04 | 44.1 ± 0.80 | 41.0 ± 1.09 |
| N75P2      | 52.0 ± 5.15 | 59.0 ± 3.34 | 179.0 ± 7.95 | 10.1 ± 0.79 | 15.24 ± 0.88 | 15.7 ± 0.39 | 408.3 ± 69.5 | 1022 ± 75.7 | 2564 ± 116 | 50.6 ± 1.20 | 44.5 ± 0.78 | 41.3 ± 1.97 |
| N100P0     | 52.4 ± 4.34 | 55.0 ± 2.17 | 192.3 ± 11.4 | 10.1 ± 1.30 | 15.37 ± 0.96 | 17.7 ± 0.61 | 460.8 ± 84.4 | 996.1 ± 108 | 3002 ± 60.2 | 49.3 ± 2.91 | 43.0 ± 1.15 | 43.0 ± 1.81 |
| N100P1     | 60.0 ± 1.78 | 55.8 ± 4.55 | 193.6 ± 7.41 | 11.4 ± 0.38 | 15.46 ± 1.35 | 16.6 ± 0.38 | 594.8 ± 38.1 | 1021 ± 110 | 2956 ± 137 | 52.7 ± 0.41 | 44.6 ± 0.87 | 39.6 ± 2.09 |
| N100P2     | 49.0 ± 2.70 | 58.0 ± 3.79 | 190.0 ± 7.39 | 9.96 ± 0.66 | 17.03 ± 1.31 | 17.4 ± 0.64 | 489.2 ± 49.1 | 1289 ± 223 | 2790 ± 255 | 49.8 ± 0.49 | 44.7 ± 0.64 | 39.6 ± 1.95 |

P > F (0.05)

|               | V4 | V6 | VT | V4 | V6 | VT | V4 | V6 | VT | V4 | V6 | VT |
|---------------|----|----|----|----|----|----|----|----|----|----|----|----|
| N             | 0.3594 | 0.5929 | 0.7519 | 0.1994 | 0.0334 | 0.8151 | 0.5926 | 0.2731 | 0.1094 | 0.5412 | <0.0001 |
| PGPR          | 0.202 | 0.314 | 0.24 | 0.0877 | 0.0812 | 0.2875 | 0.8445 | 0.3736 | 0.0658 | 0.5444 |
| N+PGPR        | 0.2273 | 0.7324 | 0.6875 | 0.5263 | 0.8936 | 0.8809 | 0.5721 | 0.3086 | 0.2403 | 0.3234 | 0.8332 | 0.3026 |

†N—nitrogen fertilizer; P0—no PGPR; P1—PGPR mixture 1; P2—PGPR mixture 2; N0, N25, N50, N75, and N100—0%, 25%, 50%, 75%, and 100% of the recommended N rate, respectively.
recommended N rate (N50P1) at the VT stage, and 100% of recommended N rate (N100P1) at the V4 stage in HP2, when compared to the other PGPR treatments evaluated using the same N rate (Table 3). Moreover, inoculation of the PGPR mixture 2 significantly increased plant height compared to the N100P0 treatment ($P = 0.0260$) at the V4 stage in HP1 (Table 2).

Stem diameter was significantly affected by N levels, especially during the later vegetative growth stages (Table 2 and Table 3). Plants grown with 50% ($P = 0.0042$), 75% ($P = 0.0050$), and 100% ($P = 0.0002$) of the recommended N rate had significantly greater stem diameter than the no N control at the VT stage during HP1, with an increase of 15.2%, 14.9%, and 18.4%, respectively. Nitrogen rate also significantly affected corn stem diameter at the VT stage in HP2, and plants with 100% of the recommended N rate had the largest stems. Although, there were no significant differences among N treatments for stem diameter at the V4 and V6 stages in HP2, there was a tendency for greater stem diameter with increasing N rates. Fancelli and DouradoNeto [49] reported that stronger stems were directly related to increased productivity since it is involved in the storage of soluble solids, which may subsequently be used in the formation of seeds. PGPR inoculations (averaged across N rates) had minimal impact on stem diameter of corn, no significant difference was observed at the V4 and VT stages and a significant decrease in stem diameter was observed at the V6 stage in HP1 (Table 2 and Table 3). However, when conducting direct comparisons between each PGPR mixture at each N level, PGPR mixture 1 at the V4 stage in HP1 tended to increased stem diameter for the no N fertilizer (N0P1) treatment, and was even significantly greater than that of the N100P0 (recommended N rate without PGPR) treatment ($P = 0.0467$).

There were no significant differences among N levels on leaf area at the V4 and VT stages for both experimental times (Table 2 and Table 3). However, average leaf area at the recommended N rate was significantly larger than that of the no N fertilizer ($P = 0.0013$) or 25% of recommended N rate treatment ($P = 0.0005$) at the V6 stage in HP1. The leaf area was not influenced by PGPR applications for both experiments (Table 2 and Table 3), while PGPR inoculations showed an increasing tendency at some N levels.

Leaf greenness (SPAD readings) was significantly affected by N levels at V6 in HP1 and at the VT stage during both experiments (Table 2 and Table 3). SPAD readings increased with increasing N rates throughout the plant growth stages; therefore, higher chlorophyll content was observed when relatively high N fertilizer rates were applied. The effects of microbial inoculations on leaf greenness varied depending on growth stage and N level for both experiments (Table 2 and Table 3).

Significant differences were observed between PGPR inoculants at the V6 and VT stages in HP1. An interaction of N level and PGPR inoculation was observed for SPAD readings at the V6 stage in HP1. A significant increase in chlorophyll content was observed after inoculation of PGPR mixtures 1 & 2 when 50% of the recommended N rate ($P = 0.0322$) was compared to the no-PGPR control at the
same N rate. When conducting direct comparisons between each PGPR mixture at each N level, PGPR mixture 1 at the V6 stage ($P = 0.0398$) and PGPR mixture 2 at the VT stage ($P < 0.0001$) had significantly greater SPAD readings than the non-inoculated control, with an increase of 4.5% and 12.3%, respectively. Moreover, PGPR mixture 1 with no N application (N0P1; direct comparison between each PGPR at each N level) had the greatest leaf area compared to other treatments, and was significantly greater than that of the N100P0 treatment ($P = 0.0183$) at the V6 stage in HP2.

The uptake of N by corn is low during early development and increases as it nears tasseling [50], which means that N generally has minimal effects on plant growth during the seedling stages. This likely is why minimal differences were observed for the plant growth parameters at the V4 stage. Moreover, another important factor that may affect seedling growth is the emergence day. Earlier emergence can lead to taller plants, greater stem diameter, and leaf area. Since temperature and sunlight duration increased from March to June; therefore, greater plant growth parameters (plant height, stem diameter, and leaf area) were found in HP2, rather than in HP1.

Overall, applying PGPR had positive effects on plant growth during the vegetative stages, especially at the V6 stage when corn plants’ need for N from soil begins to escalate. In this study, PGPR mixture 1 showed positive effects on plant height, stem diameter, and leaf greenness of corn, while PGPR mixture 2 tended to only increase leaf area and leaf greenness of corn. The difference between these two microbial inoculated mixtures may be due to the capacity of the different Bacillus spp. responses to the soil N conditions. Our results are consistent with previous studies which indicated that PGPR can increase plant height [37] [51] [52], increase plant stem diameter [53] [54], and enhance the number of leaves and leaf area of corn [55] [56]. Most of these improvements in plant growth were observed prior to the corn reaching the tasseling stage. In this study, PGPR showed more positive effects under low N soils than the soil with high rates of N fertilization. Consistently, several studies have demonstrated that when nutrient levels are high in soil, PGPR's efficacy to improve plant growth is low [12] [57] [58]. One possible reason is that the production of ethylene under low levels of nutrients could be catabolized by 1-aminocyclopropane-1-carboxylate (ACC) deaminase, produced by PGPR, to NH$_3$ and α-ketobutyrate [59]. In addition, in nutrient rich soil, plants could obtain enough N from soil by their own root absorption, thus, plants will not need rhizobacteria-enhanced N uptake.

### 3.2. Root Morphology

The influence of PGPR inoculants on root morphological parameters varied with N level and growth stage (Table 4 and Table 5). A significant N and PGPR interaction was observed for total root length at the V6 and VT stages in HP2 (Table 5), showing that PGPR inoculates had a positive effect on total root length at
Table 4. PGPR effects on corn root morphology as influenced by N rates during the V4, V6, and VT growth stages in the Horticulture Paterson Greenhouse during March to May (HP1).

| Treatment† | Total length (cm) | Average diameter (mm) | Surface area (cm²) | Total volume (cm³) |
|------------|-------------------|-----------------------|--------------------|--------------------|
|            | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    | V4    | V6    | VT    |
| NO0P       | 1879 ± 241 | 7863 ± 277 | 12877 ± 626 | 0.41 ± 0.01 | 0.60 ± 0.03 | 0.64 ± 0.04 | 238.6 ± 26.7 | 1480 ± 69.1 | 2523 ± 145 | 2.42 ± 0.24 | 22.3 ± 1.81 | 40.8 ± 4.46 |
| NO1P       | 1788 ± 323 | 7669 ± 426 | 13362 ± 221 | 0.44 ± 0.02 | 0.63 ± 0.02 | 0.59 ± 0.03 | 240.3 ± 39.8 | 1523 ± 98.4 | 2468 ± 151 | 2.58 ± 0.39 | 24.2 ± 2.11 | 36.8 ± 4.11 |
| NO2P       | 1741 ± 69.9 | 7262 ± 290 | 12475 ± 668 | 0.46 ± 0.02 | 0.63 ± 0.03 | 0.59 ± 0.02 | 252.8 ± 11.1 | 1422 ± 62.2 | 2306 ± 142 | 2.95 ± 0.25 | 22.4 ± 1.93 | 34.1 ± 2.56 |
| N25P       | 1238 ± 143 | 7115 ± 221 | 12703 ± 656 | 0.44 ± 0.02 | 0.70 ± 0.03 | 0.69 ± 0.03 | 171.1 ± 15.7 | 1548 ± 25.3 | 2720 ± 92.7 | 1.89 ± 0.14 | 26.9 ± 1.57 | 47.0 ± 2.39 |
| N25P       | 1554 ± 157 | 7738 ± 352 | 12902 ± 545 | 0.45 ± 0.01 | 0.63 ± 0.03 | 0.69 ± 0.05 | 217.2 ± 21.2 | 1539 ± 107 | 2773 ± 104 | 2.42 ± 0.24 | 24.6 ± 2.62 | 48.3 ± 4.81 |
| N25P       | 1158 ± 143 | 7503 ± 468 | 12198 ± 423 | 0.49 ± 0.02 | 0.60 ± 0.03 | 0.72 ± 0.01 | 177.8 ± 20.3 | 1394 ± 41.5 | 2755 ± 128 | 2.19 ± 0.26 | 20.8 ± 1.32 | 49.9 ± 3.00 |
| N25P       | 1608 ± 217 | 7733 ± 232 | 11582 ± 385 | 0.46 ± 0.02 | 0.61 ± 0.02 | 0.85 ± 0.04 | 229.1 ± 22.9 | 1485 ± 58.9 | 3014 ± 74.7 | 2.61 ± 0.19 | 22.8 ± 1.39 | 64.7 ± 5.10 |
| N25P       | 1333 ± 134 | 7709 ± 327 | 11337 ± 418 | 0.46 ± 0.02 | 0.63 ± 0.02 | 0.85 ± 0.08 | 190.2 ± 12.7 | 1514 ± 19.9 | 2961 ± 195 | 2.17 ± 0.07 | 23.7 ± 0.67 | 64.1 ± 10.0 |
| N25P       | 1402 ± 95.1 | 8026 ± 335 | 12462 ± 340 | 0.47 ± 0.01 | 0.61 ± 0.03 | 0.85 ± 0.03 | 208.2 ± 14.0 | 1539 ± 94.9 | 3278 ± 57.4 | 2.47 ± 0.19 | 23.7 ± 2.39 | 69.6 ± 3.22 |
| N25P       | 1777 ± 288 | 7357 ± 488 | 12960 ± 446 | 0.46 ± 0.00 | 0.65 ± 0.01 | 0.79 ± 0.02 | 258.3 ± 43.6 | 1497 ± 102 | 3176 ± 44.5 | 2.99 ± 0.52 | 24.3 ± 1.83 a† 62.6 ± 2.32 |
| N25P       | 1655 ± 148 | 6912 ± 265 | 12623 ± 311 | 0.48 ± 0.01 | 0.60 ± 0.03 | 0.81 ± 0.03 | 247.8 ± 24.2 | 1296 ± 61.2 | 3157 ± 81.7 | 2.96 ± 0.32 | 19.5 ± 1.65 ab 63.9 ± 3.78 |
| N25P       | 1268 ± 163 | 7326 ± 514 | 11979 ± 702 | 0.48 ± 0.01 | 0.55 ± 0.03 | 0.85 ± 0.05 | 189.9 ± 24.6 | 1269 ± 137 | 3166 ± 44.8 | 2.27 ± 0.30 | 17.6 ± 2.58 b 67.4 ± 3.49 |
| N25P       | 1474 ± 360 | 7544 ± 243 | 13086 ± 681 | 0.48 ± 0.01 | 0.64 ± 0.02 | 0.76 ± 0.01 | 218.9 ± 51.9 | 1503 ± 35.0 | 3094 ± 177 | 2.59 ± 0.60 | 23.9 ± 1.05 58.5 ± 3.80 |
| N25P       | 1171 ± 113 | 7434 ± 170 | 12764 ± 477 | 0.46 ± 0.02 | 0.60 ± 0.03 | 0.82 ± 0.03 | 170.9 ± 20.8 | 1397 ± 68.5 | 3245 ± 66.3 | 1.99 ± 0.30 | 21.2 ± 2.23 66.5 ± 3.68 |
| N25P       | 1503 ± 179 | 7551 ± 356 | 12950 ± 371 | 0.46 ± 0.01 | 0.63 ± 0.03 | 0.80 ± 0.02 | 216.1 ± 22.2 | 1503 ± 105 | 3237 ± 40.5 | 2.48 ± 0.22 | 24.0 ± 2.54 64.9 ± 2.14 |

|        | P > F (0.05) |
|--------|--------------|
| N      | 0.0526       |
| PGPR   | 0.416        |
| N*PGPR | 0.6569       |

†N—nitrogen fertilizer; P0—no PGPR; P1—PGPR mixture 1; P2—PGPR mixture 2; N0, N25, N50, N75, and N100—0%, 25%, 50%, 75%, and 100% of the recommended N rate, respectively. †Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at P < 0.05 among PGPR treatments under the same N rate.
Table 5. PGPR effects on corn root morphology as influenced by N rates during the V4, V6, and VT growth stages in the Horticulture Paterson Greenhouse during April to June (HP2).

| Treatment † | Total length (cm) | Average diameter (mm) | Surface area (cm²) | Total volume (cm³) |
|-------------|-------------------|-----------------------|--------------------|-------------------|
|             | V4 (cm) | V6 (cm) | VT (cm) | V4 (mm) | V6 (mm) | VT (mm) | V4 (cm²) | V6 (cm²) | VT (cm²) | V4 (cm³) | V6 (cm³) | VT (cm³) |
| N0P0        | 2438 ± 134 | 5425 ± 192 | 14458 ± 260 | 0.56 ± 0.09 | 0.58 ± 0.03 | 0.79 ± 0.03 | 431.1 ± 66.9 | 991.5 ± 73.8 | 3576 ± 111 | 6.59 ± 2.00 | 14.56 ± 1.72 | 71.4 ± 5.17 |
| N0P1        | 2568 ± 354 | 5708 ± 116 | 14080 ± 518 | 0.58 ± 0.04 | 0.70 ± 0.02 | 0.81 ± 0.06 | 481.6 ± 86.1 | 1256 ± 64.7 | 3556 ± 154 | 7.28 ± 1.61 | 22.05 ± 1.83 | 73.4 ± 8.27 |
| N0P2        | 2677 ± 173 | 5637 ± 326 | 13825 ± 648 | 0.65 ± 0.02 | 0.64 ± 0.01 | 0.78 ± 0.03 | 542.8 ± 31.1 | 1138 ± 56.0 | 3331 ± 57.8 | 8.78 ± 0.58 | 18.31 ± 0.82 | 64.5 ± 1.65 |
| N25P0       | 2581 ± 202 | 5623 ± 216 | 14270 ± 377 | 0.56 ± 0.03 | 0.63 ± 0.02 | 0.75 ± 0.03 | 457.0 ± 47.3 | 1116 ± 73.7 | 3375 ± 138 | 6.53 ± 0.98 | 17.73 ± 1.80 | 64.1 ± 4.35 |
| N25P1       | 2461 ± 64.6 | 4928 ± 262 | 13728 ± 355 | 0.59 ± 0.02 | 0.62 ± 0.03 | 0.80 ± 0.04 | 456.0 ± 12.2 | 969.4 ± 73.1 | 3447 ± 101 | 6.75 ± 0.36 | 15.30 ± 1.71 | 69.2 ± 3.56 |
| N25P2       | 2497 ± 118 | 4960 ± 297 | 13564 ± 411 | 0.63 ± 0.02 | 0.66 ± 0.02 | 0.82 ± 0.03 | 494.7 ± 37.9 | 1030 ± 76.6 | 3499 ± 87.6 | 7.83 ± 0.85 | 17.06 ± 1.68 | 72.3 ± 3.55 |
| N50P0       | 2322 ± 93.0 | 4884 ± 190 | 11981 ± 250 b | 0.60 ± 0.02 | 0.72 ± 0.02 | 0.85 ± 0.03 | 436.5 ± 25.3 | 1109 ± 46.1 | 3202 ± 111 | 6.57 ± 0.61 | 20.08 ± 1.07 | 68.4 ± 4.18 |
| N50P1       | 2487 ± 82.7 | 5321 ± 184 | 13905 ± 504 a | 0.63 ± 0.05 | 0.64 ± 0.04 | 0.82 ± 0.03 | 493.2 ± 38.9 | 1074 ± 41.2 | 3565 ± 23.9 | 7.91 ± 1.21 | 17.37 ± 1.62 | 73.2 ± 2.24 |
| N50P2       | 2343 ± 96.1 | 5135 ± 192 | 13641 ± 183 a | 0.65 ± 0.04 | 0.67 ± 0.01 | 0.79 ± 0.03 | 479.0 ± 27.6 | 1087 ± 57.9 | 3380 ± 113 | 7.89 ± 0.89 | 18.35 ± 1.34 | 67.3 ± 4.76 |
| N75P0       | 2604 ± 89.9 | 4738 ± 229 b† | 12349 ± 484 b | 0.61 ± 0.03 | 0.63 ± 0.02 | 0.85 ± 0.04 | 502.3 ± 23.3 | 939.8 ± 45.9 | 3272 ± 76.9 | 7.75 ± 0.62 | 14.90 ± 1.10 | 69.7 ± 4.63 |
| N75P1       | 2504 ± 85.3 | 4902 ± 263 ab | 12716 ± 469 b | 0.59 ± 0.02 | 0.65 ± 0.04 | 0.86 ± 0.03 | 464.4 ± 30.0 | 1014 ± 111 | 3412 ± 68.9 | 6.89 ± 0.67 | 16.87 ± 2.69 | 73.4 ± 3.58 |
| N75P2       | 2457 ± 277 | 5369 ± 138 a | 14279 ± 738 a | 0.57 ± 0.04 | 0.67 ± 0.02 | 0.91 ± 0.08 | 448.0 ± 68.3 | 1134 ± 63.0 | 3523 ± 159 | 6.56 ± 1.24 | 19.13 ± 1.68 | 67.4 ± 5.50 |
| N100P0      | 2353 ± 335 | 4519 ± 325 b | 13199 ± 219 | 0.60 ± 0.05 | 0.65 ± 0.05 | 0.81 ± 0.04 | 460.6 ± 84.7 | 940.9 ± 123 | 3360 ± 176 | 7.35 ± 1.73 | 15.90 ± 2.95 | 69.0 ± 7.35 |
| N100P1      | 3105 ± 199 | 5495 ± 177 ab | 13863 ± 431 | 0.58 ± 0.01 | 0.62 ± 0.03 | 0.84 ± 0.05 | 568.5 ± 37.8 | 1066 ± 61.3 | 3624 ± 133 | 8.29 ± 0.59 | 16.57 ± 1.68 | 77.0 ± 7.69 |
| N100P2      | 2788 ± 139 | 5934 ± 477 a | 13796 ± 509 | 0.56 ± 0.02 | 0.63 ± 0.01 | 0.85 ± 0.04 | 490.2 ± 30.9 | 1170 ± 86.9 | 3619 ± 97.2 | 6.90 ± 0.60 | 18.36 ± 1.28 | 77.0 ± 5.09 |

P > F (0.05)

N = nitrogen fertilizer; P0 = no PGPR; P1 = PGPR mixture 1; P2 = PGPR mixture 2; N0, N25, N50, N75, and N100 = 0%, 25%, 50%, 75%, and 100% of the recommended N rate, respectively. †Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at P < 0.05 among PGPR treatments under the same N rate.
relative high N levels. At the V4 stage, PGPR mixture 2 (direct comparison between each PGPR at each N level) significantly increased average root diameter by 5.6% compared to the no-PGPR control in HP1 (Table 4). The PGPR mixture 2 significantly increased total root length by 13.3% ($P = 0.0494$) and 31.3% ($P = 0.0160$) with 75% and 100% of the recommended N application rate at the V6 stage and up to 13.9% ($P = 0.0024$) and 15.6% ($P = 0.0418$) with 50% and 75% of recommended N rate at the VT stage, respectively. An increase in total root length of 16.1% ($P = 0.0013$) was observed with the inoculation of PGPR mixture 1 at the VT stage for half the recommended N rate in HP2 (Table 5). These results indicated that the selected PGPR strains in this experiment could potentially promote root growth even under N-limited conditions. Our results are consistent with those observed in several studies which have indicated that PGPR inoculations effectively increased the root length and surface area [18] [60], suggesting this resulted from PGPR synthesis of phytohormones and other secondary metabolites [61]. It is also worth mentioning that the corn hybrid used in this experiment has a high root strength (8/10) which means it has an innate capacity to grow a strong root system, which may have masked some of the potential positive effects of PGPR on root growth.

Root morphological parameters, especially total root length and root surface area, play an important role in the capture of belowground nutrient resources for plant development [62] [63] and root morphological parameters may exhibit higher water retention [64]. Several studies have reported that root structure and morphology are influenced by soil microorganisms such as rhizobacteria [52] [64] [65] [66]. El Zemrany et al. [64] investigated the root characteristics of corn where seeds were inoculated with PGPR *Azospirillum lipoferum* CRT1 during the early growth stages (for 35 days after planting, DAP) and demonstrated that plants inoculated with PGPR significantly increased root biomass, total root length, and root surface area at 26, 30, and 35 DAP. Calvo et al. [52] reported that *Bacillus* spp. mixtures could increase total root length, root surface area, root volume, and total length of fine roots of corn compared to the non-inoculated control when urea ammonium nitrate (UAN) was present at the V2 stage, while positive effects resulted when calcium ammonium nitrate (CAN) was applied at the V4 stage.

### 3.3. Biomass Accumulation and N Uptake

Significant differences were observed among N levels for biomass of roots, stems, and leaves. Plant aboveground biomass tended to increase with increasing N rate at the V6 and VT stages, no significant differences were observed at the V4 stage in both experiments (Table 6, Figure 1 and Figure 2). At the V4 stage, the no N treatment had the greatest plant biomass when compared with other N rates with the same PGPR treatment, especially in HP1. The no N control had the greatest root biomass on average (Figure 1(a)). At the V6 and VT stages, the relative high N rates (N75 and N100) had the greatest plant biomass regardless
Figure 1. Corn biomass (dry matter basis) for N rates as influenced by PGPR inoculation during (a) V4; (b) V6; and (c) VT growth stages in the Horticulture Paterson Greenhouse from March to May (HP1). Data represent means and standard errors of replicates. Within each experimental time, bar segments denoted by the same letter or with no letter assignment are not significantly different at P < 0.05 among PGPR treatments under the same N rate.

of PGPR application. The full N rate treatment increased stem and leaf biomass by 32.4% (P = 0.0124) and 39.9% (P = 0.0002) at the V6 stage and increased root, stem, and leaf biomass by 57.4% (P < 0.0001), 42.8% (P < 0.0001), and 37.9% (P < 0.0001), respectively, at the VT stage when compared to unfertilized control in HP1. An increased stem biomass of 24.8% (P = 0.02) was observed with the full N application rate at the VT stage in HP2 (Figure 2(c)). Plants with 50% and
Figure 2. Corn biomass (dry matter basis) for N rates as influenced by PGPR inoculation during (a) V4; (b) V6; and (c) VT growth stages in the Horticulture Paterson Greenhouse from April to June (HP2). Data represent means and standard errors of replicates. Within each experimental time, bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$ among PGPR treatments under the same N rate.

75% of the recommended N rate also showed significant increases in root, stem, and leaf ($P < 0.0001$) biomass compared to unfertilized control at the VT stage, which was similar to plant biomass of the full rate treatment. Although there were no significant responses to application of the PGPR mixtures on biomass accumulation at some growth stages, corn seeds inoculated with PGPR mixtures had similar or greater plant biomass when compared to non-inoculated seeds under the different N levels during the growing period (Table 6, Figure 1 and
Table 6. Analysis of variance results for biomass of root, stem, and leaf at the V4, V6, and VT stages and N concentration of root, stem, and leaf at the VT stage in the Horticulture Paterson Greenhouse during March to May (HP1) and April to June (HP2).

| Source        | P > F (0.05) | HP1       | Leaf       | HP1       | Stem       | Leaf       |
|---------------|--------------|-----------|------------|-----------|------------|------------|
|               |              | Root      | Stem       | Leaf      | Root       | Stem       | Leaf       |
| Root biomass  |              |           |            |           |            |            |            |
| N             | 0.0215       | 0.5443    | 0.3490     | 0.9068    | 0.6927     | 0.1424     |
| PGPR          | 0.9223       | 0.6643    | 0.8420     | 0.7681    | 0.8807     | 0.8916     |
| N*PGPR        | 0.4215       | 0.3304    | 0.6177     | 0.7303    | 0.5150     | 0.5974     |
| Stem biomass  |              |           |            |           |            |            |            |
| N             | 0.1483       | 0.0132    | 0.0001     | 0.3483    | 0.9717     | 0.0972     |
| PGPR          | 0.3891       | 0.4976    | 0.3520     | 0.0713    | 0.0075     | 0.0903     |
| N*PGPR        | 0.0113       | 0.0164    | 0.0486     | 0.6981    | 0.3664     | 0.2314     |
| Leaf biomass  |              |           |            |           |            |            |            |
| N             | <0.0001      | <0.0001   | <0.0001    | <0.0001   | <0.0001    | <0.0001    |
| PGPR          | 0.3464       | 0.1729    | 0.5295     | 0.479     | 0.3985     | 0.6479     |
| N*PGPR        | 0.3480       | 0.4435    | 0.7611     | 0.4965    | 0.9811     | 0.5311     |
| N concentration |            |           |            |           |            |            |            |
| N             | <0.0001      | <0.0001   | <0.0001    | <0.0001   | <0.0001    | <0.0001    |
| PGPR          | 0.7112       | 0.6267    | 0.2639     | 0.7130    | 0.9031     | 0.8717     |
| N*PGPR        | 0.1660       | 0.3340    | 0.6033     | 0.0547    | 0.6315     | 0.6244     |

Figure 2). Both treatments inoculated with PGPR mixtures had greater stem biomass than the non-inoculated control, increasing 21.8% and 22.9% with PGPR mixtures 1 ($P = 0.0264$) and 2 ($P = 0.0151$), respectively, at the V6 stage in HP2. The improvement of plant biomass by PGPR was only observed at the V6 stage, but not at the V4 and VT stages (Table 6, Figure 1 and Figure 2). The lack of PGPR effects on plants evaluated at the V4 stage may be due to the low rate of biomass accumulation and nutrient uptake during the early corn growth. In contrast, no significant difference between non-PGPR and PGPR treatments on biomass accumulation evaluated at the VT stage may be due to the small amount of nutrients provided by PGPR could not satisfy the high nutrient requirements during the late vegetative growth stage. Nitrogen and PGPR interactions were observed for plant biomass accumulation at the V6 stage in HP1 (Table 6). PGPR mixture 1 with no N fertilizer (N0P1) had the greatest root, stem, and leaf biomass at the V6 stage, although there were no significant differences observed compared to the non-inoculation control, an increase of 34.8% ($P = 0.0339$), 63.0% ($P = 0.0202$), and 41.3% ($P = 0.0283$) occurred when compared to PGPR mixture 2, respectively. PGPR mixture 2 with 50% of recommended N (N50P2) had the greatest stem and leaf biomass with an increase of 34.4% ($P = 0.0461$) and 25.6% ($P = 0.0495$) compared to the N50P0 treatment.
at the V6 stage, respectively. However, at 75% of recommended N rate, inoculation of PGPR strains had no benefit on aboveground biomass accumulation, even showed lower stem and leaf biomass than the no-PGPR control at the V6 stage. These results indicated that PGPR inoculation induced an increase of plant biomass that was slightly greater than the non-PGPR treatment at the different N levels, especially with low or half-rate N application.

Plant tissue N concentrations were significantly different among N treatments, with N concentrations tending to increase with increasing N rate regardless of whether the PGPR inoculants were added or not at the VT stage for both experimental times (Table 6 and Figure 3). Plants receiving 75% and the full N rate had significantly greater root, stem, and leaf N concentration compared to 25% of recommended N rate and unfertilized control, while the half N rate treatments also significantly increased plant tissue N concentrations compared to the unfertilized control (Figure 3).

Figure 3. Nitrogen concentration (%) in root, stem, and leaf at different N rates as influenced by PGPR inoculation at the VT stage in the Horticulture Paterson Greenhouse from March to May (left) and from April to June (right). Fertilizer rates are percentages of the 100% rate (135 kg N ha⁻¹) recommended by Alabama Cooperative Extension System for corn on a Coastal Plain soil. Data represent means and standard errors of replicates.
Plant tissue N concentrations compared to the unfertilized control are shown in Figure 3. These results indicated that under a properly managed greenhouse condition that prevented nutrient loss through leaching, 50% or 75% of the recommended N rate could satisfy plant N requirements during the vegetative growth stages, which may mask the positive effects of inoculated PGPR strains [67]. No significant differences were observed for the response of corn N concentrations to PGPR inoculation (Figure 3). Also, there was no N and PGPR interaction observed for plant tissue N concentrations (Table 6). However, PGPR applications resulted in equivalent or greater plant tissue N concentrations compared to non-PGPR treatments under low N level conditions, while a slightly lower plant tissue N concentration was observed when PGPR inoculations were combined with relative high N rates. This may be due to the dilution effect from greater plant tissue biomass. The results of leaf N concentration were consistent with the results of SPAD readings (Table 2 and Table 3) due to the high positive correlation between these two parameters [52] [68] [69]. These results indicated the capacity of PGPR to improve NUE of corn under N limited conditions and a potential for increased corn yield. Generally, the Bacillus spp. strains could increase N uptake by various mechanisms, such as producing phytohormones, solubilizing soil nutrients, enhancing root growth (root length and surface area) for nutrient absorption [70] [71] [72].

In our experiment, PGPR mixture 1 had a greater effect on increasing plant biomass accumulation under conditions where no N was added, while PGPR mixture 2 had a greater benefit in increasing plant biomass accumulation with half the recommended N rate. Both microbial inoculants had a tendency to improve plant tissue N concentrations. Our results for plant biomass and N concentration were consistent with previous studies that have shown the positive effects of PGPR inoculation on plant dryweight and N uptake of corn [26] [30] [47] [72] [73]. Biari et al. [73] indicated that inoculation of PGPR strains can increase corn growth parameters, such as plant height and shoot dry weight and also enhance grain dry weight and seed quality (100-seed weight and nutrients content). Therefore, PGPR treatments in our experiment that enhanced plant growth parameters and biomass accumulation could lead to a potential increase in corn yield. In addition, these positive effects of PGPR are mainly attributed to its capacity to promote better absorption of essential nutrients that are responsible for the high rate of photosynthesis [52] [73]. Consistently, a stronger root system, greater SPAD reading and dry weight biomass were observed with PGPR application in our experiment.

4. Conclusion

Overall the selected PGPR mixtures applied with half the recommended N rate promoted corn growth and produced corn biomass and tissue N concentrations equal to or greater than that of the full N fertilization rate under greenhouse conditions. The high amounts of N fertilization may have masked the potential
effect of PGPR inoculations, especially in the late growing stages of corn. Therefore, PGPR inoculants should be considered as tools that will complement nutrient efficiency practices by increasing the plant’s nutrient uptake efficiency, thereby reducing N losses and reducing the amount of applied N. Further studies are needed in order to determine the threshold of N fertilization reduction that could be achieve when PGPR inoculants are applied to different crops and with different types of nitrogen fertilizers, as well as investigate the optimal field management practices for simulating the efficacy of PGPR under field conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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