Utilization of Poultry Litter and Plant Growth-Promoting Rhizobacteria to Improve Crop Productivity

by

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Abstract

In order to satisfy the demand for food on a global scale, more than 180 million tons of chemical fertilizers are applied annually to increase crop yields. However, the consequences of indiscriminate chemical fertilizer use are a significant environment problem leading to soil degradation which may negatively affect several soil ecological functions. There are also strong indications that phosphorus supplies used for fertilizers are limited, indicating a need to more efficiently utilize and recycle phosphorus back into agricultural soils. Poultry litter and plant growth-promoting rhizobacteria could be used as alternatives to chemical fertilizers. If utilized appropriately, they are environmental-friendly and can contribute towards the promotion of plant growth and crop yield. Thus, the main aim of this dissertation was to evaluate the impact of poultry litter and plant growth-promoting rhizobacteria use on plant growth and productivity under different environmental conditions and management practices. This dissertation consists of five parts: (1) Effect of poultry litter on grain yield and crop productivity in a wheat-soybean double-cropping system; (2) Effect of nitrogen fertilization on winter canola yield and nitrogen uptake; (3) Effect of PGPR on corn growth under different fertility sources; (4) Effect of PGPR on corn growth at various nitrogen rates; and (5) Effect of PGPR on corn growth under drought stress. In the first study, wheat and soybean yield were evaluated in a two-year field experiment. Greater wheat yields were observed when both poultry litter and inorganic N were applied, indicating that fertilization practices combining poultry litter and inorganic N may be an alternative to just applying inorganic chemical fertilizer for the growth and productivity of

wheat. The increased soybean grain yield observed under double cropping suggests that a combination of poultry litter and inorganic N fertilizer use for a wheat-soybean cropping system could provide sustainable yield production. In the second study, plant biomass, grain yield, and N uptake of winter canola increased with increasing N application rates, with optimal yields occurring when 197 to 232 kg N ha^{-1} was applied to these southeastern US soils. Moreover, applying both poultry litter and inorganic N fertilizer to agricultural fields could reduce the dependence on solely using chemical fertilizers without decreasing winter canola yields, thereby providing sustainable yield production for winter canola in the southeastern US. In the third study, the selected PGPR strains improved plant growth parameters and biomass accumulation in the early growth stages of corn and the performance varied with different fertility sources. However, applying N at recommended rates may have masked the influence of PGPR on corn growth. Thus, PGPR inoculation with different N rates was evaluated in the fourth study, and showed that the selected PGPR strains with a half-dose of N fertilization could promote corn growth and produce corn biomass and N concentration equal to or greater than that of uninoculated full N fertilization rate. In the fifth study, the selected PGPR strains increased corn root morphology, thereby improving aboveground growth and biomass accumulation of corn under water stress. Overall, utilization of poultry litter and plant growth-promoting rhizobacteria could improve plant growth and produce biomass or grain yield comparable to standard fertilization practices and potentially reduce some of the dependency on chemical fertilizer usage.

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List of Abbreviations

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
B	Boron
BS	Biosolid
C	Carbon
Ca	Calcium
CAN	Calcium ammonium nitrate
Cu	Copper
DAP	Days after planting
EC	Electrical conductivity
ES	Effect sizes
EVS	E.V. Smith Research Center-Field Crops Unit
Fe	Iron
HP	Horticulture-Paterson Greenhouse Complex
IAA	Indole-3-acetic acid
IF	Inorganic fertilizer
ISR	Induced system resistance
K	Potassium
MA	Meta-analysis
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NUE	Nitrogen use efficiency
P	Phosphorus
PAU	Prattville Agricultural Research Unit
PGPR	Plant growth-promoting rhizobacteria
PL	Poultry litter
PSRC	Plant Science Research Center
RR	Response ratio
RSR	Root shoot ratio
S	Sulphur
SRL	Specific root length
UAN	Urea ammonium nitrate
Zn	Zinc

1. Literature Review

1.1 Plant Growth Promotion by PGPR

Soil-microbe-plant interactions are complex and are known to influence plant health and productivity. Plant and bacteria interactions in the rhizosphere play an important role in the transformation, mobilization, and solubilization of the limited nutrient pool in soil (Jeffries et al. 2003). Some soil bacteria can thrive in the niche around plant roots by root exudates and lysates. Population densities of these bacteria in the rhizosphere can be up to 100-fold higher than that in bulk soil and up to 15% of the root surface may be covered by a variety of different bacterial strains. While these bacteria utilize the released nutrients from the host plant for their growth, they also secrete metabolites into the rhizosphere.

Bacteria inhabiting the rhizosphere that are beneficial to plants are classified as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1980). These include strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Bashan and de-Bashan 2005). PGPR are capable of aggressively colonizing plant roots and promoting plant growth by producing and secreting various chemical regulators around the rhizosphere (Fig. 1). Such mechanisms through which PGPR are beneficial to plants include: 1) enhancing asymbiotic nitrogen fixation; 2) solubilizing inorganic phosphate and mineralization of organic phosphate and other nutrients; 3) increasing ion uptake, such as iron, zinc and other essential micronutrients; 4) producing plant growth regulators or phytohormones such as indole-acetic acid (IAA), ethylene, cytokinins and gibberellins; 5) producing siderophores and antagonistic effects against phytopathogenic microorganisms by the synthesis of antibiotics, enzymes or

fungicidal compounds; and 6) modulating plant development, such as ACC (1-aminocyclopropane-1-carboxylic acid) deaminase elicitors (Zafar et al. 2012).

1.1.1 Nitrogen Fixation

Nitrogen (N) is the most commonly deficient nutrient for plant growth and productivity. Although the atmosphere is about 78% N_2, this form of nitrogen is relatively unavailable to plants. Atmospheric N_2 can be converted into plant-utilizable forms by biological N_2 fixation via N-fixing microbes through a complex enzyme system known as nitrogenase (Kim and Rees 1994). These N_2-fixing microbes are generally categorized as 1) symbiotic N_2-fixing bacteria and 2) non-symbiotic (free living, associative and endophytic) N_2-fixing forms.

Symbiotic N_2-fixing bacteria infect and establish symbiotic relationships with the roots of leguminous plants involving a complex interaction between hosts and symbiotic bacteria for nodules colonization (Giordano and Hirsch 2004). The most studied and exploited PGPR is rhizobia, because of its ability to fix nitrogen for host legumes (Fred et al. 1932). For instance, N_2-fixing bacteria such as *Rhizobium* and *Bradyrhizobium* can form nodules on roots of leguminous plants such as soybean (*Glycine max* (L.) Merr.), pea (*Pisum sativum* (L.)), peanut (*Arachis hypogaea*) and alfalfa (*Medicago sativa*), in which they convert N_2 into ammonia, which can be used by the plant as a nitrogen source (Leinhos and Bergmann 1995). There is a great deal of contradictory information on legume-rhizobia symbioses (Vessey 2003), so it is not reviewed here.

In recent decades, researchers have begun to pay more attention to associative N_2 fixation in a vast array of non-legume crop plants. For example, the N_2-fixer *Azospirillum* can fertilize wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor*), and corn (*Zea mays* L.) where yield increases can be attributed to increased root development and thus increased water and mineral

uptake (Oken et al., 1998). Boddey et al. (2001) indicated sugarcane (*Saccharum officinarum* L.) routinely obtains 20-60% of its N requirements from the associative N_2-fixing rhizobacteria. In particular, Sevilla et al. (2001) found *Gluconacetobacter diazotropicus* significantly contributes to sugarcane N content under controlled conditions. Moreover, associative N_2-fixing bacteria need only small amounts of fixed N compared to their hosts, which is used for plant growth.

1.1.2 Ion Uptake

Poorly soluble inorganic nutrients, such as phosphorus (P), often limit plant growth due to its poor plant availability. Insoluble phosphorus is widely present in soil as an inorganic mineral (such as apatite) or as one of several organic forms (including inositol phosphate, phosphotriesters, and phosphomonesters), while plants only absorb P in two soluble forms, as monobasic ($H_2PO_4^-$) and dibasic (HPO_4^{2-}) ions. To overcome P deficiency in agricultural fields, abundant phosphatic fertilizers are frequently applied. However, most soluble P fertilizers are rapidly converted into insoluble complexes in soils. This is not only costly but also environmentally undesirable.

In this context, microorganisms with P-solubilizing abilities could increase the supply of plant-available P forms in soil and hence may be a potential substitute or compliment for chemical fertilizers. Phosphate solubilizing microorganisms, like *Azotobacter*, *Bacillus*, *Pseudomonas*, *Microbacterium*, *Enterobacter*, and *Rhizobium* (Bhattacharyya and Jha 2012), are common in the rhizosphere. Previous studies identifying and evaluating microorganisms with P solubilizing associations include *Bacillus circulans* and *Cladosporium herbarum* with wheat (Singh and Kappor 1999), *Pseudomonas chlororaphis* and *P. putida* with soybean (Cattelan et al. 1999), *Rhizobium* sp. and *Bradyrhizobium japonicum* with radish (*Raphanus sativus* L., Antoun et al. 1998), *Pseudomonas fluorescens* with corn (Krey et al. 2011), *Burkholderia* sp. with lupin

(*Lupinus* L., Unno et al. 2005), *Pseudomonas* sp. with greengram (*Vigna radiata* (L.), Ahemad and Khan 2012), *Rhizobium* sp. with lentil (*Lens culinaris*) in fungicide-applied soil (Ahemad and Khan 2011), and *Pseudomonas fluorescens* and *Enterobacter radicincitans* sp. with corn and oilseed rape (*Brassica napus* L., Krey et al. 2011).

Typically, P-solubilizing bacteria synthesize low molecular weight organic acids that mobilize phosphorus by means of ionic interactions with the cations of phosphate salts, and secrete a variety of different phosphatases for releasing phosphate groups bound to organic matter and catalyzing the hydrolysis of phosphoric esters (Hayat et al. 2010). Besides providing P to plants, they also augment plant growth by stimulating the efficiency of biological N_2-fixation, enhancing plant stress tolerance to drought, salinity and metal toxicity.

Although iron (Fe) is one of the most abundant minerals on Earth, in the soil it is relatively unavailable for direct assimilation by microorganisms. In aerobic soils, iron mainly occurs as a constituent of oxyhydroxide polymers with extremely low solubility at about 10^{-18} M when pH is neutral, while minimal concentrations of iron required for normal growth of plants range from 10^{-9} to 10^{-4} M, and for the optimal growth of most soil microbes are approximately 10^{-5} to 10^{-7} (Dobbelaere et al. 2003). To overcome this problem, soil microorganisms secrete low-molecular weight iron-binding molecules (siderophores) that bind Fe^{3+}, transport it back to the microbial cell, and then make it available for microbial growth. Siderophores are specific Fe (III)-chelating agents that not only can adsorb Fe^{3+} to meet plants' iron requirements, but also make the chelated iron unavailable to pathogenic microorganisms, which leads to improved plant health (Khan et al. 2009). Siderophore production is very common among *Pseudomonas*, *Frankia*, and *Streptomyces* (Hayat et al. 2010), which may contribute significantly to an increased mobility of Fe in soil and within the rhizosphere in particular, making it more available for plants.

1.1.3 Phytohormone Production

Plant growth-promoting rhizobacteria synthesize and export phytohormones that may play a regulatory role in plant growth and development. Phytohormones, also known as plant growth regulators, are organic substances that alter the principal mechanism of growth regulation and cell differentiation in plants at extremely low concentration. Many bacterial species are capable of producing auxin or ethylene, or both, and synthesis of gibberellins and cytokinins has been documented.

The physiologically most active auxin in plants is indole-3-acetic acid (IAA), which is known to stimulate both rapid and long-term responses in plants. Generally, IAA secreted by rhizobacteria interferes with the many plant developmental processes because the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted from soil bacteria (Glick 2012). IAA produced by rhizobacteria likely interfere with the physiological processes of plants (e.g. enhance RNA and protein synthesis and stimulate cell division) by changing the plant auxin pool. Moreover, bacterial IAA increases root surface area and length, and thereby provides the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Glick 2012).

It has been estimated that 80% of bacteria isolated from the rhizosphere can produce IAA including PGPR species. IAA production have been well documented with the following, *Rhizobium leguminosarum*, *Azotobacter* sp., *Pseudomonas fluorescens*, *P. tolaasii*, *Mycobacterium* sp., *Bacillus circulans*, *B. magaterium*, and *B. subtilis* (Hayat et al. 2010). All these bacteria species were efficient in IAA production and significantly increased growth of

different types of plants [e.g. wheat, rice (*Oryza sativa* L.), canola (*Brassica napus* L.), corn, and spinach (*Spinacia oleracea* L.)] (Hayat et al. 2010).

Cytokinins are another important type of phytohormone usually present in small amounts of biological samples. The effects of exogenously applied cytokinins on plants are numerous, the most notable of which is enhanced cell division, but also root development and root hair formation are reported. Plants and plant-associated microorganisms have been found to contain over 30 growth-promoting compounds of the cytokinin group, and some reports indicate that as many as 90% of the microorganisms found in the rhizosphere are capable of releasing cytokinins when cultured *in vitro*. Cytokinin production has been observed in *Azotobacter* sp., *Azospirillum* sp., *Rhizobium* sp., and *Paenibacillus polymyxa* (Dobbelaere et al. 2003).

Gibberellins are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue. Only a few studies showed the evidence of gibberellins production by PGPR. Gutierrez-Manero et al (2001) first found that four types of gibberellins are produced by *Bacillus pumilus* and *B. licheniformis*. In addition, gibberellin production also has been demonstrated in *Azotobacter* sp., *P. polymyxa*, *Rhizobium leguminosarum* bv. phaseoli, *A. brasilense*, *A. lipoferum*, *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *B. pumilus*, and *B. licheniformis* (Dobbelaere et al. 2003).

1.1.4 ACC Deaminase Synthesis

Ethylene is the only gaseous phytohormone, and is an essential metabolite for the normal growth and development of plants. Under stress conditions such as those generated by salinity, drought, water logging, heavy metals and pathogenicity, the endogenous level of ethylene is significantly increased which negatively affects overall plant growth. However, PGPR could possess the enzyme ACC deaminase, which facilitates plant growth and development by

decreasing ethylene levels, thereby inducing salt tolerance and reducing drought stress in plants (Ahemad and Kibret 2013). Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera including *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium* etc. (Ahemad and Kibret 2013). Such rhizobacteria take up the ethylene precursor ACC and hydrolyze it into ammonia and 2-oxobutanoate (or α-ketobutyrate). The degradation of this compound creates an ACC concentration gradient between the interior and exterior of the plant, and finally makes a reduction of the internal ethylene level. Besides, ACC deaminase in combination with auxins causes a considerable effect on important physiological processes such as root system development. The IAA synthesized by PGPR that is bound to the surface of either the seed or root could be taken up by the plant and in conjunction with endogenous plant IAA production, may either stimulate plant cell proliferation or elongation. Alternatively, IAA production can stimulate the activity of the enzyme ACC synthase to convert S-adenosylmethionine to ACC (Glick et al. 1998).

1.1.5 Indirect Positive Effects

The major indirect mechanism of plant growth promotion by rhizobacteria is by acting as biocontrol agents (Glick 2012). In general, competition for nutrients, niche exclusion, signal interference, induced systemic resistance (ISR) and antifungal metabolite production are the chief modes of biocontrol activity by PGPR. Many PGPR have been reported to modify plant cell wall structure and synthesize proteins and chemicals involved in plant defense mechanisms. Lipopolysaccharides, siderophores and salicylic acid are the major determinants of PGPR-mediated ISR (Ramamoorthy et al. 2001). Moreover, ISR involves jasmonate and ethylene

signaling within the plant and these hormones stimulate plant defense responses against a variety of plant pathogens (Glick 2012).

Azospirillum, *Azotobacter*, *Bacillus*, *Enterobacter*, *Paenibacillus*, *Pseudomonas*, and *Streptomyces* have been reported as a potential genera of rhizobacteria that can reduce pathogen infections (Bhattacharyya and Jha 2012). Among these PGPR, *Pseudomonas* has been identified as the best-characterized biocontrol agent. For instance, *P. fluorescens* strain WCS417r can elicit systemic disease resistance in plants (Pieterse et al. 2001). Another study indicated that *P. fluorescens* strain WCS374 suppresses *Fusarium* wilt in radish leading to a 40% yield increase (Bakker et al. 2007).

1.1.6 Application of PGPR in Agronomy

Researchers in India and the former Soviet Union conducted widespread tests during the early to mid-20th century, in order to study the effects of PGPR on different crops. These tests showed that the use of PGPR resulted in a 50 to 70% yield increase (Cooper 1959; Brown 1974). These field experiments provided clues concerning the optimal conditions for bacterial colonization and growth promotion of target crops, though during this time an understanding of the detailed mechanisms of plant growth promotion by rhizobacteria was largely nonexistent. However, great variations in the plant response to PGPR in laboratory and field studies (Table 1), especially *Azotobacter*, *Pseudomonas*, *Bacillus*, *Enterobacter*, and *Azospirillum* are the most widely reported.

Many studies support the idea that increases in plant nutrient uptake are largely due to changes in the root system and morphology, and more specifically, increased root length and root surface area. For example, the addition of *Pseudomonas*, *Bacillus*, *Enterobacter*, *Azospirillum* or other PGPR, to tomato (*Solanum lycopersicum*), canola, wheat (Hall et al. 1996), potato

(*Solanum tuberosum*, Kloepper et al. 1980), corn (Dobbelaere et al. 2001), rice (Alam et al. 2001), and some other plants (Hamaoui et al. 2001; Lucy et al. 2004) have resulted in larger root systems with an increase in root length and root weight. Among these genera, *Bacillus* and *Pseudomonas* have been the most highly studied in the past decade because of their high N fixation and P solubilization ability.

Moreover, PGPR can also induce physiological changes in plants through the synthesis of specific enzymes and plant growth regulators. For example, ethylene plays a critical role in various developmental processes that regulate node factor signaling and nodule formation and also have a primary function in plant defense systems. Many studies have reported that when plants have been inoculated with PGPR, ethylene production increases as a result (Khan et al. 2009). In addition, plant growth regulators, such as IAA, produced by *Azospirillum*, have shown significant growth-promoting abilities in wheat and pearl millet (*Pennisetum glaucum* (L.), Barbieri and Galli 1993). Furthermore, the addition of IAA to soil could enhance the uptake of iron and other elements (zinc, calcium, etc.) in plant roots (Lippmann et al. 1995). However, the reasons for the positive impacts of PGPR on plant growth and productivity are not fully understood, and no consistent results have been identified as to what is the best fertilization and field management approach to optimize the stimulatory effects of PGPR under field conditions. Therefore, research is necessary to determine the full potential of rhizobacteria to promote plant growth and to identify the most optimal management practices using PGPR such as:

What fertilization rates are needed to maximize crop yield when PGPR is applied?

Does manure applications improves PGPR growth promotion efficiency?

Will PGPR improve crop growth during drought stress?

1.2 Review of Poultry Litter Utilization in Agronomy Using Meta-Analysis

Since the last half of the twentieth century, broiler chicken (*Gallus gallus domesticus* L.) production has experienced rapid expansion worldwide with the United States being by far the largest producer, marketing more than 8.5 billion birds annually (USDA-NASS 2015). Consequently, a significant amount of poultry litter (PL) is being generated, totaling more than 12.8 million tonnes in 2015 (1.5 kg litter/broiler chicken; USDA-NASS 2015; Mitchell and Tu 2005). More than two-thirds of broilers produced in the US are produced in five southeastern states (Georgia, Alabama, Arkansas, North Carolina, and Mississippi), and as a result PL is abundant in this region. The most cost-effective and environmentally safe way to use this waste is to land-apply it as a nutrient source. Currently, more than 90% of the PL generated is being used as a nutrient source (Moore et al. 1995); however, it has primarily been applied repeatedly on pastures and hayfields within short distances of broiler production facilities due to convenience and lower transportation costs (Moore et al. 1995).

In recent years, interest in using PL as a low-cost alternative to inorganic fertilizer (IF) sources has increased among row crop producers because of PL availability coupled with the potential for improving crop production. Poultry litter contains all the nutrients essential for plant growth and has an approximate 3-3-2 (N-P_2O_5-K_2O) fertilizer grade equivalent (Mitchell and Donald 1995). Application of PL to cropland can also increase soil organic matter (Watts et al. 2010a), thereby improving soil quality and productivity (Kingery et al. 1994). Thus, PL may be a valuable nutrient source for row crop production systems. Numerous studies have shown that PL is an effective fertilizer source (Reddy et al. 2004; Wiatrak et al. 2004; Mitchell and Tu 2005; Hirzel et al. 2007; Tewolde et al. 2009a; Watts and Torbert 2011). Poultry litter may also produce yields equivalent to, or greater than, those of IF sources. For instance, Mitchell and Tu (2005) reported that no differences in relative yield were observed when broiler litter was applied

at the same total N rate as ammonium nitrate. Hirzel et al. (2007) found similar results with corn silage; yield averages obtained from PL additions were comparable to those of urea. Watts and Torbert (2011) also reported that soybean yield was increased 8 out of 9 years when PL was used as a nutrient source compared to inorganic fertilizer application. However, PL use efficiency and its effect on crop productivity varies among these studies. Thus, there is a need for a comprehensive review to determine specifically the extent of PL's influence on crop production.

The efficacy of PL applications to enhance crop growth (yield and nutrient uptake) depends upon PL nutrient availability. Nutrient availability from PL is influenced by many abiotic and biotic factors including soil pH, soil texture, tillage methods, application time, and application rate. In addition, it is often difficult or impossible to fully account for, or control, all soil variables in an experimental design in greenhouse or field experiments. As a result, there is a poor understanding of how the interactions between PL, soil properties (soil texture and soil pH), and environmental and management conditions, influence crop productivity, suggesting the need to elucidate the causative mechanisms affecting nutrient availability from PL and its impact on crop productivity.

Considering the desire to use PL as an alternative nutrient source for row crops, and the many sources of heterogeneity that may occur in an agricultural production system that may influence experimental conditions (different crops, soil texture, soil pH, tillage techniques, and application methods, etc.), a comprehensive quantitative meta-analysis (MA) is needed to determine which management practices optimize forage and crop production. In agronomy, MA has generally been used to compare the effects of cropping systems or cropping techniques on yield or biomass production (Miguez and Bollero 2005; Miguez et al. 2008; Rusinamhodzi et al. 2011; Biederman and Harpole 2013; Liu et al. 2013).

Meta-analysis performed for this literature review was based on the principles described by Hedges et al. (1999) with all data being analyzed using the metafor R-package (Viechtbauer 2010). To determine the influence of PL vs. IF on crop yield, a natural log of response ratio (RR) was used to calculate effect sizes (ES) as $\text{ES} = ln\ (\text{RR}) = ln\ (\frac{\bar{Y}_{PL}}{\bar{Y}_{IF}})$, where *ln* is the natural logarithm, and $\bar{Y}_{PL}$ and $\bar{Y}_{IF}$ are the means of crop yield in the PL and IF treatments, respectively. Response ratio is the most common metric of effect size used in plant ecology meta-analysis (Hedges et al. 1999; Koricheva and Gurevitch 2014) and log transformations are needed to maintain symmetry with this analysis (Hedges et al. 1999; Borenstein et al. 2009). The full statistical analysis was described by Lin et al. (2017).

By applying the selection criteria, 85 citations from the literature and five unpublished studies were retained for inclusion in the MA, while some categories within the analysis contained as few as two independent studies (> 10 observations). Analyses were conducted on natural logs of the response ratio of crop yield and the magnitude of the summary effect was checked using a Z-test (Borenstein et al. 2009) to determine whether it statistically differed from zero, i.e., the response ratio differing from unity.

1.2.1 Effect of PL on Crop Yield

An evaluation of homogeneity for the entire dataset determined that there was a significant difference in effect size (Q = 26784, I^2 = 96 %, df = 865, $P < 0.0001$). This indicated that there was heterogeneity when all studies were grouped together, thus warranting the need for further analysis by introducing categorical variables. However, there were no significant differences ($P = 0.36$) between effects of PL and IF applications to soils on crop production, therefore further tests were performed aimed at partitioning the underlying sources of variation to determine which factors had the greatest influence on crop yield. Heterogeneity statistics were calculated

for each categorical summary effect (Table 2). All response variables evaluated resulted in low or moderate heterogeneity ($11.0 \leq I^2 \leq 52.6\%$). Between-group heterogeneity analysis was conducted for each categorical variable representing the subgroups described in Table 2. For each categorical variable other than PL application method, the *P*-value was less than 0.05, which means that there are significant differences within each subgroup, so each variable was analyzed one-by-one.

Soil texture and pH

The effect of PL vs. IF on crop production in relation to soil texture is displayed in Fig. 2. A significant increase in yield was observed for the loam-, sandy loam-, and silty clay loam-textured soils when PL is applied instead of IF, while a significant negative relationship (lower yield than IF) was observed in both the sand and silty clay-textured soils. No differences were observed with the clay loam and silt loam soils. The greatest benefit of PL additions on crop production was obtained in silty clay loam soil, increasing crop yield by 10.1%. In contrast, PL application had 36.4% lower crop yields in sand soils when compared to IF. A low N availability in PL combined with a considerable N loss (ammonia volatilization and nitrate leaching) in sand soils (Shepherd and Bhogal 1998) maybe the reason for lower yield. Therefore, the plant, especially short-season crops like annual grasses, would be less competitive with weeds (Woodard and Sollenberger 2011) and would have a lower yield than for IF application. Given that only one study (< 10 observations) was conducted in clay, silt, loamy sand, or sandy clay loam soil (one study for each soil), the influence of these soil textures on crop productivity responses to PL application were not evaluated. Soil texture reflects the particle size of a soil and influences the soil's ability to conserve water and nutrients. Soil texture as an important soil physical property and is also interconnected with soil chemical and biological properties. The

increased productivity of loamy soils receiving PL additions reported from this analytical review could be a result of higher soil water-holding capacity and improved soil structure which promotes aggregation (Revell et al. 2012; Ndor et al. 2015).

Yield responses increased with decreasing soil pH when PL was applied (Fig. 3). Thus, increases in crop yield occurred with PL additions to acidic soils, whereas no positive response was observed in neutral or slightly alkaline soil and a significant negative effect was observed when the pH was greater than 7. The relationship between initial soil pH (x) and effect size of yield (y, the natural log of ratio of PL application yield to IF application yield) was $y = 0.135 - 0.0238x$ (SE_b (standard error of slope) = 0.011; $P = 0.025$). Generally, PL is treated with an acidifying agent (litter amendments for NH_3 control in broiler rearing facilities) and has a pH near 7, while litter that is not treated has a pH near 8 or slightly higher (Blake and Hess 2001). One hypothesized mechanism by which PL applications to soil affect crop productivity is through a liming effect, resulting in increased soil pH (Kingery et al. 1994; The et al. 2006; Revell et al. 2012). Therefore, PL application can increase the pH of acidic soil, which is beneficial to plant growth. Kingery et al. (1994) evaluated the impact of long-term (15-28 years) broiler litter applications on soil properties and found that soil pH increased 0.5 unit to a depth of 60 cm under soils receiving PL compared to non-litter. Crop growth can be constrained by low availability of Ca, Mg, P, and K in acidic soils (Glaser et al. 2002). When the soil pH is increased, P and K availability to plants increases and Al toxicity decreases (Steiner et al. 2007; Chan et al. 2008).

Tillage system

Crop response to PL additions varied with tillage system (Fig. 4). Poultry litter had a significantly greater positive effect under conservation tillage (strip-till or no-till) than for

conventional tillage situations, compared to IF. No-till has been shown to increase organic matter retention and cycling of nutrients in soils. As a result, crop yield increased 11.03% with PL when compared to inorganic fertilizer application. Differences among tillage systems may have also been related to the influence of increased soil cover resulting from winter cover crops or crop residues. However, due to the limited number of studies evaluated on winter cover crops and the amount of crop residue left on the soil prior to fertilizer application, an in-depth analysis to determine the relationship among tillage systems and crop residues was not conducted.

Soil tillage is a soil preparation method used to provide a suitable seedbed for sowing or transplanting, and minimize compaction to optimize crop growth. In this MA, only the common tillage methods were evaluated. Other methods such as ridge-till, mulch-till, and reduced-till were not evaluated due to a limited number of observations. Our analysis showed that applying PL to strip-till and no-till systems had a significant positive effect on crop production. There have been numerous studies showing the benefits of conservation tillage practices in the US. These practices can substantially reduce soil erosion, increase soil moisture, improve soil structure, increase soil organic matter, and increase the retention level of macronutrients (P, K, Ca, and Mg) and micronutrients (Mn, Zn, and Cu), which all result in increased crop yields (Edwards et al. 1988; Edwards et al. 1992; Nyakatawa et al. 2001; Wiatrak et al. 2004; Schomberg et al. 2011; Watts and Torbert 2011). Poultry litter and other manure source additions have also been shown to increase pH, organic matter, N, P, K, C, Ca, and Mg; enhance water retention; and decrease bulk density in soil (Arriaga and Lowery 2003; Sistani et al. 2010b; Watts et al. 2010; Adeli et al. 2011). Therefore, the combined effects of no-till or strip-till with PL application can greatly improve soil condition and soil health, which most likely contributed to the higher yields being observed.

Application rate

Our analysis indicated that there was a positive trend for PL application rate on crop yield (Fig. 5). For example, crop yield increased with increasing application rates (up to 30 Mg ha^{-1}) when compared to IF at the same N rate. The relationship between PL rate (x) and effect size of yield (y) was y = -0.0402 + 0.0057x (SE_b = 0.001; $P < 0.0001$). Application rates were dependent on the initial soil nutrient levels, crop requirement, and the available N in PL. Therefore, PL application rates were converted to total N rate and a similar positive linear relationship between N rate and crop yield was observed. In this MA, most of the individual experiments compared PL application to inorganic nitrogen fertilizer (93%; including urea, urea-ammonium nitrate, anhydrous ammonia, ammonium nitrate, and nitrate). The relationship of the nitrogen rate ratio of PL to IF (x) and effect size of yield (y) obtained from these individual studies confirmed the results, where y = -0.0696 + 0.0643x (SE_b = 0.014; $P < 0.0001$). Crop yield increased with increasing PL application rates. This was to be expected given that most crops respond to increasing N rates. This review also showed that, although PL is an organic material, the release of available N via mineralization continues to increase with application rate. Not only is N increased with higher PL application rates, but P, K, Ca, Mg and the other essential nutrients are also added to soil, potentially improving plant growth, which may lead to higher crop yield.

Application time

There was a statistically significant effect of PL application on crop productivity between groups as categorized by PL application time (Fig. 6). For this analysis, of the 866 total observations, 37 tested the residual effects (no fertility source applied) on crop productivity in the year after the cessation of PL application, and the successive years were up to 4 years. The

residual (carryover of PL nutrients from the previous year) influence of PL application resulted in slightly greater crop yield when compared to residual of IF application, with effect size for yield equaling 0.0429 ± 0.0622 (P = 0.4907). Although there were no significant differences between the residual of PL and IF, averaged across 4 years, the residual of PL increased the crop yield above the residual of IF by 4.4%. There were relatively few observations, so residual effects were not analyzed by years, while the residual effects were greatest in the first year and declined in successive years. These results were observed for silage maize (Hirzel et al. 2007), cotton (*Gossypium spp.*, Tewolde et al. 2016), rye (*Lolium multiflorum* L.) and grain corn (Nyakatawa et al. 2001) crops. This is a result of the PL nutrients being primarily in organic form; thus, the nutrients may have a carryover effect for succeeding years (Edmisten et al. 1992). This suggests that the residual PL has important implications for subsequent crop productivity and can reduce the use of fertilizer for succeeding years (Hirzel et al. 2007).

Significantly higher crop yields were observed for PL applications occurring more than 30 d before sowing (BS), whereas no positive effects where observed for PL applied either less than 10 d or 30 d BS. This indicates that applying PL less than 10 d or within one month before sowing had no difference on crop yield compared to standard IF application, while applying PL one month or more before sowing may provide more time for the mineralization of organic compounds of the litter to optimize nutrient availability in soil for uptake by plant roots.

Application time was also grouped into spring application vs. fall application. Some studies have indicated that spring application had greater economic return for corn and soybean yields (Randall and Vetsch 2005), but Jn-Baptiste et al. (2012) observed no differences in grain yield and plant nutrient uptake when spring was compared to fall application. Analysis of this study showed an opposite result (not mentioned in the figures), as fall application had a positive effect

on crop yield with 6.85% increase, but was not significant ($P = 0.06$). Therefore, compared with spring application of PL, fall application may be an option and may offer the advantage of saving time during the busy planting season for farmers (Ruiz Diaz and Sawyer 2008). However, the environmental implications of P loss from fall-applied PL must be taken into consideration when making this decision.

Application method

Fertilizer application method had little effect on crop yield (Fig. 7). Subsurface band application of PL (placing PL in narrow bands below the soil surface) at the same total N rate was shown to have the highest crop yield, albeit not significantly different from IF. Although slightly greater crop yield was observed with surface broadcast application of PL compared to IF, it was not significant. In contrast, slightly lower crop yield was observed with surface incorporation of PL, when compared to that of IF with the same application method, but it was not significant. It is also important to note that PL application method was generally associated with a particular tillage system. For instance, surface broadcast and subsurface band application was associated with no-tillage or strip tillage, while broadcast incorporation was associated with conventional tillage. Due to the limited studies obtained from our literature search for both tillage techniques vs. the PL and IF application methods, we did not analyze the interactions between those management strategies. Band placement of fertilizer and liquid manure slurry has been shown to increase nutrient use efficiency and crop yield by placing the fertility source near the plant, while traditional broadcast application practices evenly apply the nutrients to the entire field (Bittman et al., 1999; Pote et al., 2009; Tewolde et al., 2009b; Blackwell et al., 2010; Watts et al., 2011; Ma et al., 2013). Similar to the banding of IF or manure slurry, subsurface band application of PL places the litter where plant roots can easily absorb nutrients for crop growth,

potentially increasing yields as well. However, subsurface band application equipment for PL is still in the developmental stages and not commercially available for widespread use. It is also important to note that subsurface band application of PL data collected for this study were taken from studies evaluated under conservation tillage (strip-till or no-till), which may have also contributed to increased crop yield.

Crop type

The influence of PL in relation to inorganic fertilizer on yield from a range of different crop species (major crops) was shown in Fig. 8. Significantly higher crop yields were observed for corn, cotton, soybean, and peanut from addition of PL to soil when compared to IF, while lower yields were observed for bermudagrass hay (*Cynodon dectylon* (L.) Pers.) production. No statistically significant effects were observed for silage maize, rice, and wheat when PL was compared to IF; fescue (*Festuca arundinacea*) and rice showed particularly variable effects regarding yield. Overall, the average yield of all crops where PL was applied tended to have equal or slightly lower yields compare to when IF was applied. Common row crops (corn and soybean) had the highest yield with PL addition when compared to IF, while winter crops and hay fields (wheat and bermudagrass) tended to have lower yields with PL application.

Poultry litter has been widely evaluated on different types of crops, including cereals, oilseed crops, grasses, fruits, vegetables and melons. In this MA, we collected data from 33 crop types; only 10 of them had an increased response to PL application in multiple studies (Fig. 8). Soybean showed the greatest response to PL application with 14.4 ± 2.4% increase, presumably as a result of macro- and micronutrient additions. Soybeans can form nodules to fix N for plant growth. Thus, N is not a limited nutrient for soybeans. However, P, Fe, Mo, and other micronutrients are necessary to maximize root nodulation. For example, when P in the soil is not adequate, it can

lead to a deficiency in N uptake (Cassman et al. 1980). Of 866 total observations, 244 observations were from corn. A 5.8% corn grain yield increase was observed, indicating that PL application may be used as an alternative nutrient source to IF for corn production. The only significant negative effect of PL application found was for bermudagrass. There are two probable reasons why bermudagrass performs differently to fescue and silage maize. First, bermudagrass needs about 23 to 34 kg N per cutting; and PL application could not satisfy the average 114 kg N requirements needed per growing season (four cuttings, Lee et al. 2013). Secondly, inorganic N is usually applied after each cutting, while PL is usually applied only once per growing season. There were some other grasses included that showed a lower yield (only one study for each of these grasses, so that they were not included in the meta-analysis). Moreover, a positive effect was observed on yield of these grasses only with the high PL rates (Sleugh et al. 2006), or where PL application occurs multiple months before planting (Warman and Cooper 2000), or repeated applications occur over multiple years (Maguire et al. 2008). This may be a result of PL's slow mineralization capacity not being able to satisfy N requirements for the short growing periods and the overall high N requirements for the grasses.

Study duration

For all observations, crop yield had a slightly negative response (not significant) to the addition of PL when compared to IF. This difference can be attributed to nutrient availability between the two fertility sources. For example, N from most IF sources is available at the time of application or a few weeks later. On the other hand, when PL is applied, N is released slowly over time and it may take multiple years before all of it becomes available. Therefore, differences in crop yield may be different depending on whether PL is applied only one year or for multiple years. In this review, we observed that crop yield increased with duration (years of

repeating the application) of PL application (up to thirteen years, Fig. 9); however, the number of observations decreased with the increasing study duration. The relationship between study duration (x) and effect size of yield (y) was y = -0.0576 + 0.0196x (SE_b = 0.002; $P < 0.0001$). More than 50% of the experiments were conducted for only one or two years and these experiments showed slight negative effects on crop yield. When continuous yearly PL applications to the same soil reached three years, the long-term benefits of PL on crop productivity became apparent compared with continuous IF application.

Generally, crop nutrient and soil fertility management mainly depends upon the complex long-term integrated approach rather than the short-term one. This MA showed that crop productivity had a positive response with the duration (years of repeated application) of PL application (Fig. 9). Higher crops yields were observed from studies with repeated yearly PL applications. For example, an 8.2% yield increase was observed when PL was applied to the same soil for 10 years when compared to IF with the same duration. Similarly, Diacono and Montemurro (2010) reviewed the effects of organic amendments in long-term studies (3 – 60 years) and found that crop yield increased up to 250% when high rates of municipal solid waste compost was applied. Unlike inorganic N, PL contains high levels of organic N and slow mineralization leads to less N availablity for crops at the beginning of PL applications. On average, only 55% of the organic N mineralized and 75 % of total N is available in the first year after application (Castellanos and Pratt 1981; Moore et al. 1998) with the remaining N tied up in organic matter being released over succeeding cropping years (Edmisten et al. 1992). Therefore, when PL is repeatedly applied for multiple years, organic N, P, and other plant nutrients accumulate in the soil and are mineralized slowly into inorganic form for plant uptake in subsequent years.

1.2.2 Effect of PL on Nutrient Uptake

Crop nutrient concentration is determined by plant uptake and depends on the level of available nutrients in soil (Pederson et al. 2002). Several studies have reported on the influence of PL on plant uptake of primary nutrients (N, P, and K), while only a limited number of studies have reported data on secondary macronutrient and micronutrient uptake. Thus, only plant nutrient uptake for N, P, and K was synthesised in this MA. Given that plant nutrient concentrations can vary depending on the vegetative part of the plant being evaluated, plant tissue nutrients were analyzed separately by leaf, stem, reproductive part, and whole plant, based on data collected from the reviewed studies. Results of the MA show that applying PL to soil significantly affected plant nutrient concentrations and nutrient uptake (Figs. 10 and 11).

Nitrogen (N)

Plant N concentrations from PL studies for leaf N ($P < 0.0001$), stem N ($P = 0.0027$), and reproductive N (reproductive parts including flower and grain, $P = 0.2915$) was less than that observed with IF application. Since N concentration in leaves and stems gradually decreases over the course of the growing season, most researchers generally focus on grain N concentration (Tewolde et al. 2007). The significant negative response of leaf and stem N to increased PL application rate was likely due to inorganic N being readily available at the time of application, while PL must first be mineralized. It is also important to highlight that, for this MA, approximately 72% of the leaf, stem, and reproductive N observations were collected from cotton, and analysis of the cotton data had smaller leaf and stem N effect sizes compared to other crops. Thus, the results of PL effects on cotton leaf and stem N concentration may have largely impacted the MA for plant leaf and stem N concentrations.

When the whole plant was analysed for N concentration (e.g. forage and some vegetable crops), no significant effect was observed between PL and IF additions ($P = 0.5904$). A negative response was observed for plant N uptake (primarily forages in this analysis) when comparing PL to IF ($P < 0.0001$), which also contributed to slightly lower plant N concentrations for PL application. Generally, litter-N exists in both organic and inorganic forms. The soil inorganic fraction is usually in the ammonium form, which may constitute 10 to 60% of the total litter-N (Chadwick et al. 2000; Collins et al. 1999) that is readily available for plant absorption in the ammonium form or after conversion to nitrate. The organic fraction of litter-N is found in the form of proteins, nucleic acids, and other organic compounds derived from plant or animal tissues, which may constitute 40 to 90% of the total litter-N (Chadwick et al. 2000b; Collins et al. 1999); this becomes available only after mineralization via soil microbial activity (Ma et al. 1999). Therefore, it is difficult to predict how much N from the organic fraction will be available for plant uptake and utilization during the growing season. For example, litter-N availability through mineralization may be influenced by field conditions (e.g., soil properties and weather) and management strategies such as PL application time and method, tillage, and study duration (Schjønning et al. 1999; Gordillo and Cabrera 1997; Watts et al. 2007; Watts et al. 2010b). In the present MA study, a synthesis of N concentrations of plant tissues from litter-derived N as influenced by abiotic factors experienced under different field conditions and management practices was not conducted due to the limited number of studies available.

Phosphorus (P)

Poultry litter application significantly increased leaf ($P < 0.0001$), stem ($P < 0.0001$), and reproductive ($P = 0.0365$) P concentrations compared to IF with the same total N rate. The effect size was generally small and observed mainly in row crops, including corn, cotton, soybean,

barley (*Hordeum vulgare*), and peanuts; some instances were also noted in vegetable and fruit crops. Plant P concentrations for whole plants were significantly higher with PL ($P < 0.0001$) compared to IF. Among the 92 plant P observations, 66 were grasses (including corn silage, sorghum, sudangrass (*Sorghum* x *drummondii*), turfgrass, bermudagrass, fescue, and forage-mix), which largely influenced the effect size for plant P concentrations. Greater plant P uptake was observed in PL treatments compared to IF, increasing by 14.77% ($P = 0.0002$). This synthesis shows that applying PL could improve plant P uptake and increase tissue P concentration in both row crops and grasses compared to IF. These results are consistent with reviews that focused on the effect of manure on forage production (Pant et al. 2004). In this MA, PL application rates were based on crop N requirements, so P inputs from PL were likely higher than plant P requirements (Bolan et al. 2010). These results show that, although litter-P was applied in excess of P requirement and IF was applied based on the recommended rates, plant tissue P concentrations were higher with PL additions.

Potassium (K)

Among K concentration analyses, stem and reproductive K effect size variables contained only 13 observations and most were obtained from cotton plants; thus, these two effect size values largely depended on cotton K concentration. There was no difference between IF and PL application on leaf ($P = 0.1092$), stem ($P = 0.5574$), and reproductive ($P = 0.8063$) K concentrations; whereas, there was a significant positive response to PL application for whole plant K concentration ($P < 0.0001$) and K uptake ($P < 0.0001$). Half of the 40 observations for K uptake were collected from corn studies; thus, corn's capacity to absorb K from soil may have largely influenced average K uptake results. Approximately 85% of plant K concentration data were collected from forages (including bermudagrass, corn silage, fescue, turfgrass, and forage-

mix). Both composted and fresh PL treatments produced higher forage K than inorganic N, P, and K treatments (Warman and Cooper 2000; Warren et al. 2008; Wood et al. 1993) and showed a positive linear response in tissue K concentration with litter application (Warman and Cooper, 2000). The slightly higher K concentration in plants receiving PL indicates that K concentration in PL can satisfy plant growth requirements, mainly due to K in manure being mostly in inorganic forms so that virtually all K is available for plant uptake. Therefore, this MA suggests that using PL as a nutrient source for agricultural crops can provide appreciable quantities of the essential plant nutrients N, P, and K.

Secondary nutrients (Ca, Mg, S)

Poultry litter is also a source of secondary nutrients such as calcium (Ca), magnesium (Mg), and sulphur (S). Our results showed lower leaf Ca ($P = 0.5846$) but higher plant Ca concentrations ($P = 0.5368$) with the addition of PL compared to IF (Fig. 11). In this MA, cotton studies largely contributed to the leaf Ca concentration data, while most of the plant Ca concentration data were collected from forages. Thus, opposite responses (leaf Ca < 0 and plant Ca > 0) between the leaf and plant Ca concentrations may be due to differences in requirements and absorption capacity between the plant species. Calcium is an essential macronutrient for plant cell wall development and metabolism and comprises 0.1 to 5% of the plant fresh weight, while PL contains 14 g Ca kg^{-1} on average (Moore 1998). Therefore, PL applied to Ca deficient soils may improve soil Ca concentrations, thereby increasing tissue Ca concentration. Magnesium concentration response to PL application varied depending on the plant tissue part. Magnesium concentrations were significantly higher in reproductive parts ($P = 0.0072$), while no differences were observed in crop leaves ($P = 0.0718$), stems ($P = 0.1360$), and whole plant concentrations ($P = 0.3095$) when compared to IF application (Fig. 11). In the studies reviewed

for the MA, Mg was not applied when IF was used, while PL additions supplied the soil with Mg. As a result, the addition Mg with PL contributed to higher Mg concentrations in plant reproductive tissues.

Micronutrients (B, Fe, Mn, Cu, Zn)

Poultry litter application can also influence plant micronutrient concentrations (Fig. 11). Due to the limited number of studies evaluating the response of PL applications on plant micronutrient concentrations (especially those partitioned in the different plant tissues), this MA only analysed leaf and whole plant micronutrient concentrations. There was a significant positive response in leaf B ($P < 0.0001$) and significant negative responses in leaf Fe ($P = 0.0186$) and Mn ($P = 0.0001$) when comparing PL to IF. Leaf Cu ($P = 9680$) and Zn ($P = 0.0629$) concentrations were not affected by the addition of PL compared to IF. Furthermore, PL did not change plant Cu, Fe, and Zn concentrations ($P = 0.3441$, 0.6684, and 0.3119, respectively) when PL and IF application rates were based on the same total N rate. Since plants require B in very small quantities, supplying the soil with B by PL addition could significantly influence plant B concentrations. Poultry litter contains small amounts of these nutrients; thus, the increase of micronutrient concentrations in plant tissues may be due to improved soil quality following PL additions. For example, changes in soil pH and organic matter and addition of extra micronutrients with PL application can affect macronutrient availability.

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Table 1. Examples of PGPR tested on various crop types.

PGPR	Plant	Conditions	Results of addition of PGPR to plant	Reference
Pseudomonas sp. PS1	greengram	pots	Significantly increased plant dry weight, nodule numbers, N and P uptake, seed yield and seed protein	Ahemad and Khan, 2010, 2011, 2012
Bacillus sp. PSB10	chickpea	pots	Significantly improved growth, nodulation, chlorophyll, leghaemoglobin, seed yield and seed protein	Wani and Khan, 2010
Pseudomonas, *Baccillus*, *Azotobacter*	corn	pots	Plant height increased 17.15% and dry weight increased 35.48% compared to control	Jarak et al., 2012
Bacillus, *Pseudomonas*, *Paenibacillus*	wheat spinach	pots	Increased IAA production, plant height 25%, 37%, leaf area 47%, 49%, dry weight 54%, 53%, respectively	Cakmakci et al, 2006
Azospirillum amazonense	rice	pots	Grain dry matter (11.6%), number of panicles (18.6%), and N uptake (18.5%) increased	Rodrigues et al., 2008
Azospirillum chroococcum, *A. lipoferum*	cotton	fields	Seed yield (21%), plant height (5%), and microbial population in soil (41%) increased	Anjum et al., 2007
Berkholderia sp. *Bacillus* sp.	barley	pots	Increased root weight up to 16.7% and shoot weight up to 347%	Canbolat et al., 2006
Pseudomonas sp.	soybean, wheat	fields	Significantly increased soil enzyme activities, total productivity, nutrient uptake	Sharma et al., 2011
Pseudomonas corrugate	corn	fields	Crop N, P content, yield (45%), grain dry weight (94%), root-shoot ratio (278%) increased	Kumar et al., 2007

Table 2. Grouping categorical variables and studies included in the meta-analysis.

Grouping	Sub-heading	Number of studies	$P_{Z\text{-test}}$	I^2	$P_{moderator}$
Soil texture	Sand	4	< 0.0001	21.81	< 0.0001
	Sandy loam	26	0.0187		
	Loam	3	0.0009		
	Silt loam	28	0.6817		
	Silty clay loam	5	0.0040		
	Silty clay	3	0.0002		
	Clay loam	7	0.3416		
Tillage system	Conventional (CT)	25	0.0196	11.04	< 0.0001
	Strip (ST)	2	0.0060		
	No-till (NT)	8	< 0.0001		
Application method	Surface broadcast	32	0.5457	28.46	0.5102
	Surface incorporation	44	0.2378		
	Subsurface banding	7	0.4569		
Application time	Before sowing (BS) ≤ 10 d	65	0.5402	52.55	0.0082
	10 d < BS ≤ 30 d	17	0.0845		
	BS > 30 d	9	0.0037		
Crop type	Corn	29	< 0.0001	35.44	< 0.0001
	Cotton	17	0.0139		
	Forage-bermudagrass	5	< 0.0001		
	Forage-fescue	3	0.4695		
	Forage-silage maize	7	0.1821		
	Peanut	2	0.0200		
	Rice	3	0.1925		
	Soybean	5	< 0.0001		
	Wheat	3	0.9194		

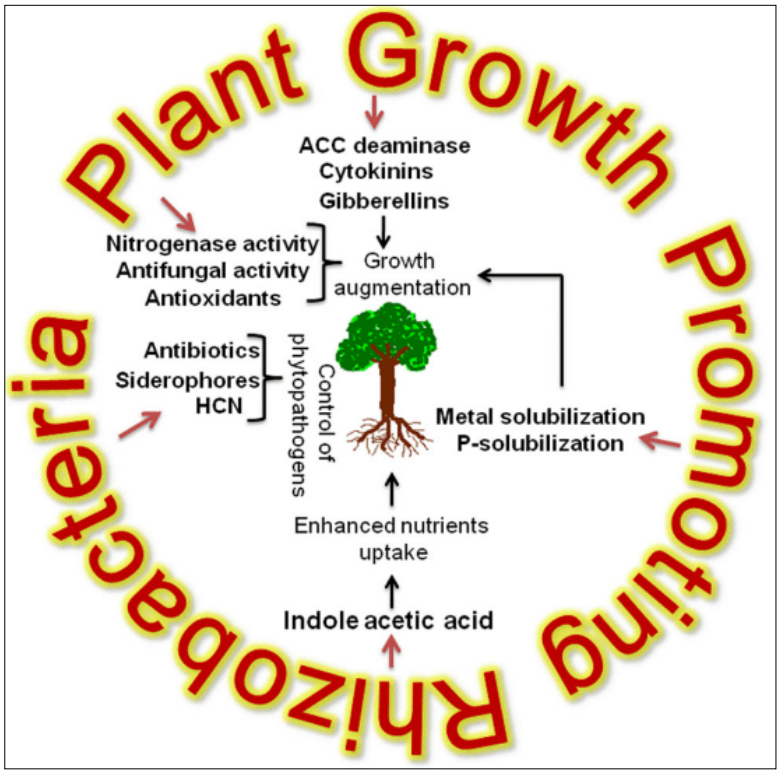

Fig. 1. Mechanism of plant growth promotion by rhizobacteria (Ahemad et al. 2014).

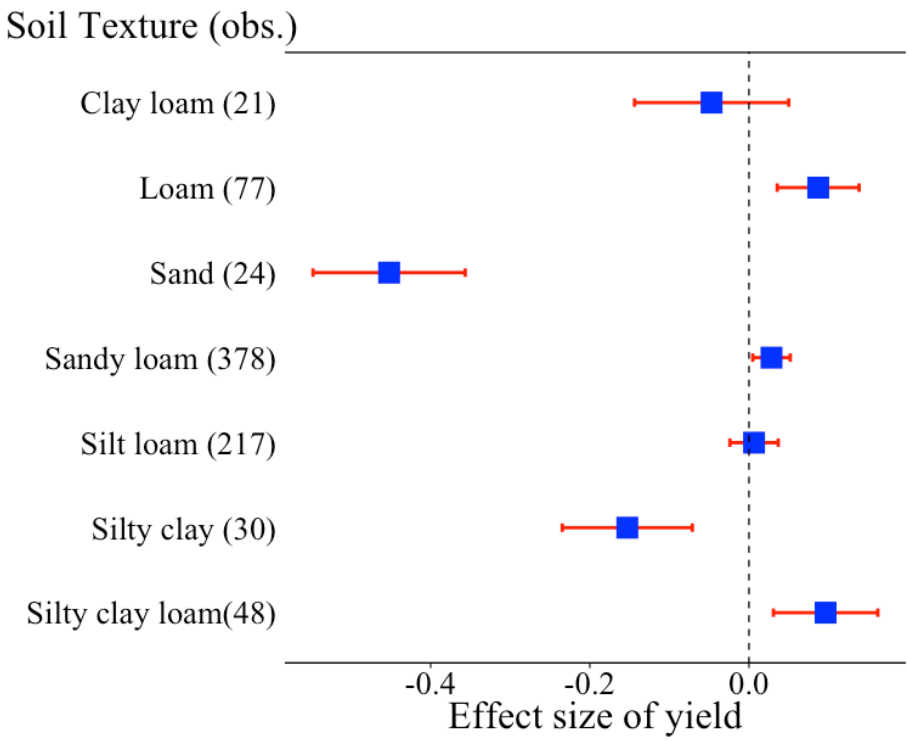

Fig. 2. Response of crop productivity to poultry litter application expressed as the average effect size. The dotted vertical line represents the null hypothesis [ln(RR) = 0], the blue squares are the point estimates of effect size, and the horizontal red lines are the associated 95% confidence intervals for the population parameter.

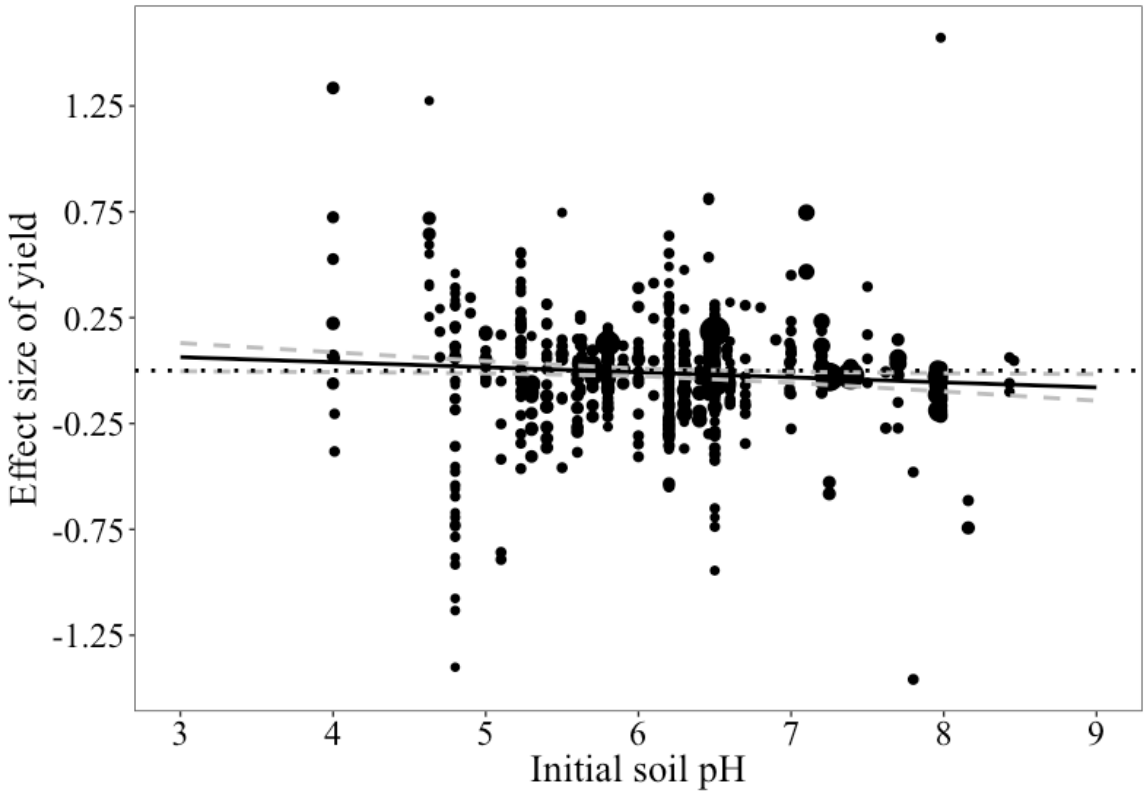

Fig. 3. Meta-regression analysis for response of crop yield to poultry litter application at various soil pH. Data points are sized in proportion to the inverse of their standard error. The dotted horizontal line represents the null hypothesis [ln(RR) = 0], the solid line is the regression line, and the dashed lines are the 95% confidence intervals of the linear regression.

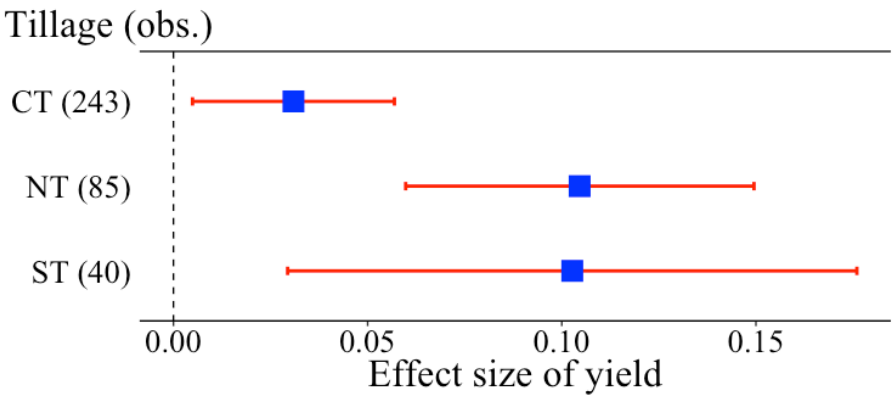

Fig. 4. Response of crop productivity to poultry litter application expressed as the average effect size. The dotted vertical line represents the null hypothesis [ln(RR) = 0], the blue squares are the point estimates of effect size, and the horizontal red lines are the associated 95% confidence intervals for the population parameter. CT, conventional tillage; NT, no-till; ST, strip-till.

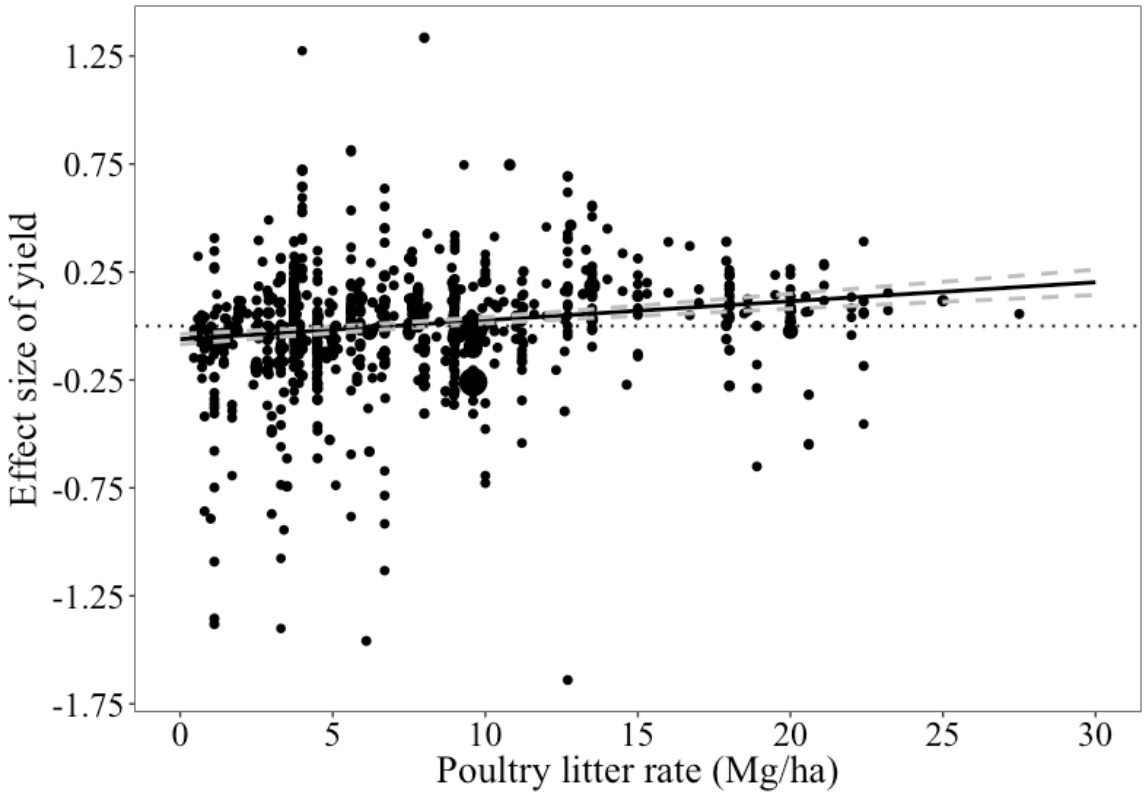

Fig. 5. Meta-regression analysis for response of crop yield to poultry litter application at various application rates. Data points are sized in proportion to the inverse of their standard error. The dotted horizontal line represents the null hypothesis [ln(RR) = 0], the solid line is the regression line, and the dashed lines are the 95% confidence intervals of the linear regression.

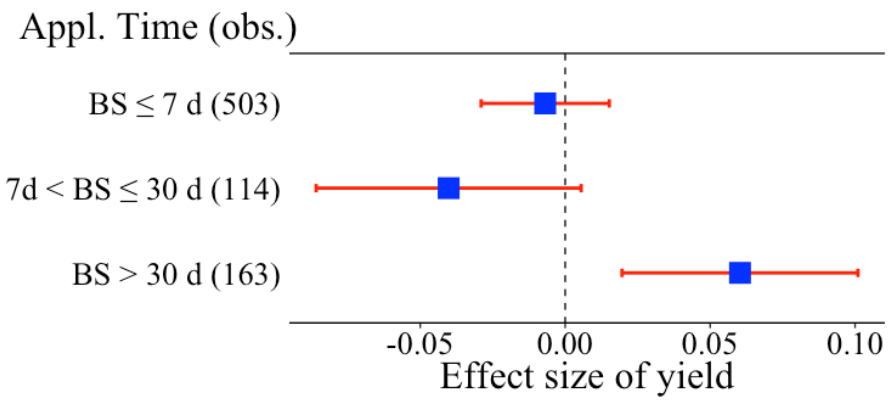

Fig. 6. Response of crop productivity to poultry litter application expressed as the average effect size. The dotted vertical line represents the null hypothesis [ln(RR) = 0], the blue squares are the point estimates of effect size, and the horizontal red lines are the associated 95% confidence intervals for the population parameter. BS, before sowing.

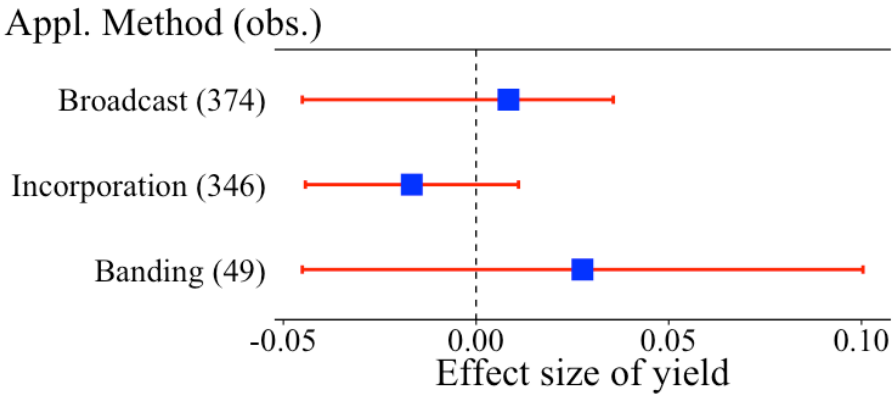

Fig. 7. Response of crop productivity to poultry litter application expressed as the average effect size. The dotted vertical line represents the null hypothesis [ln(RR) = 0], the blue squares are the point estimates of effect size, and the horizontal red lines are the associated 95% confidence intervals for the population parameter.

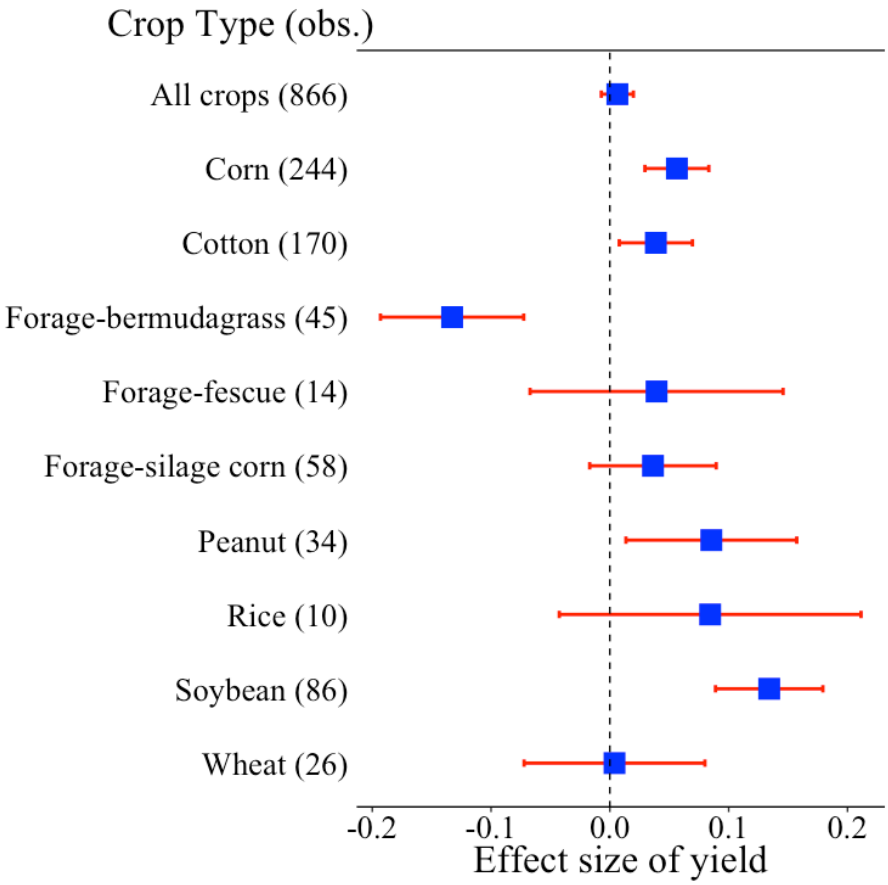

Fig. 8. Response of crop productivity to poultry litter application expressed as the average effect size. The dotted vertical line represents the null hypothesis [ln(RR) = 0], the blue squares are the point estimates of effect size, and the horizontal red lines are the associated 95% confidence intervals for the population parameter.

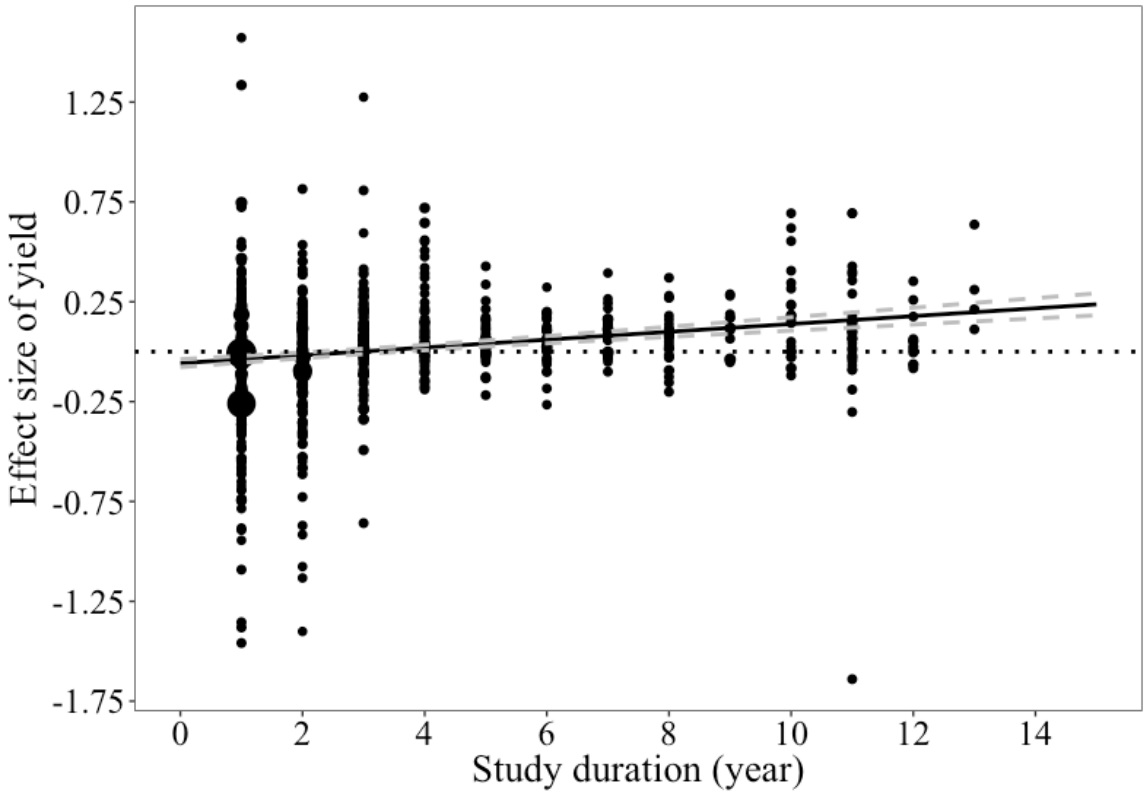

Fig. 9. Meta-regression analysis for response of crop yield to poultry litter application at various study durations. Data points are sized in proportion to the inverse of their standard error. The dotted horizontal line represents the null hypothesis [ln(RR) = 0], the solid line is the regression line, and the dashed lines are the 95% confidence intervals of the linear regression.

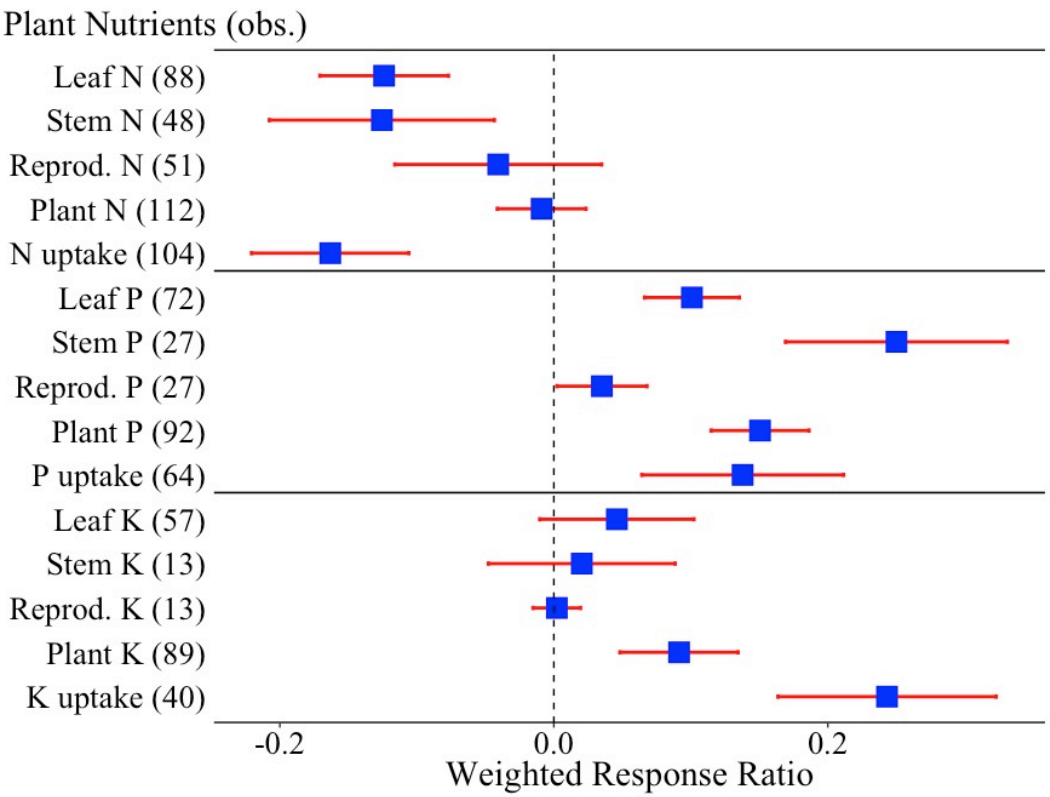

Fig. 10. Response of plant primary macronutrients to poultry litter additions expressed as the weighted response ratio of PL and IF with 95% confidence intervals. The dotted vertical line represents the null hypothesis [ln(RR) = 0], the blue squares represent the point estimates of effect size, and the horizontal red lines represent the associated 95% confidence intervals for the population parameter.

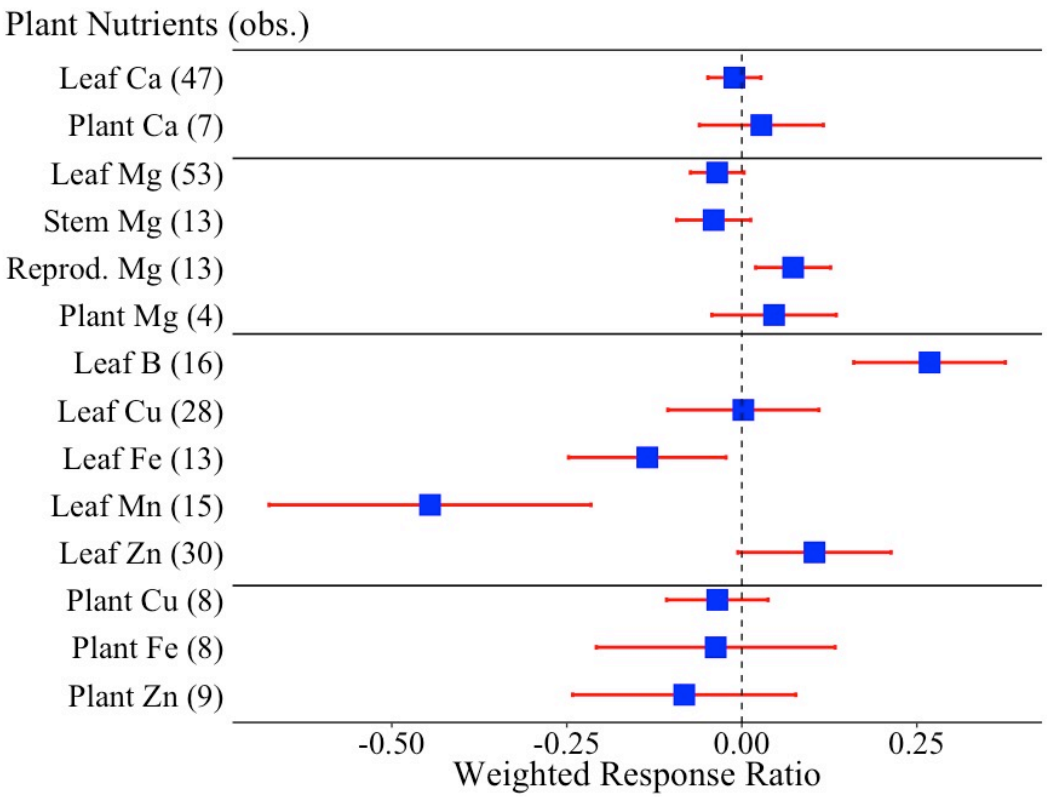

Fig. 11. Response of plant macro- and micronutrients to poultry litter additions expressed as the weighted response ratio of PL and IF with 95% confidence intervals. The dotted vertical line represents the null hypothesis [ln(RR) = 0], the blue squares represent the point estimates of effect size, and the horizontal red lines represent the associated 95% confidence intervals for the population parameter.

2. Effect of Poultry Litter on Grain Productivity under Wheat-Soybean Double-Cropping Systems

2.1 Abstract

Poultry litter (PL) application and double cropping are management practices that could be used with conservation tillage systems to increase yields compared to conventional monocropping systems. The objective of this study was to evaluate winter wheat (*Triticum aestivum* L.) and soybean [*Glycine max* (L.) Merr.] yield response of PL verses inorganic N fertilizer applications under a double cropping system. A two-year field study was conducted at two locations on a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult) and a Lucedale fine sandy loam (fine-loamy, siliceous, subactive, thermic Rhodic Paleudult). At each location, the experimental design was a randomized complete block with six treatments replicated four times. Fertility treatments for winter wheat in the first year included an unfertilized control (P_0N_0), inorganic N fertilizer (135 kg ha^{-1}, P_0N_{135}), PL at the rate of 45 kg N ha^{-1} plus 90 kg ha^{-1} inorganic N ($P_{45}N_{90}$), PL at the rate of 90 kg N ha^{-1} plus 45 kg ha^{-1} inorganic N ($P_{90}N_{45}$), and PL at the rate of 135 kg N ha^{-1} ($P_{135}N_0$). In the second year, winter wheat in all plots received inorganic N. Wheat-soybean double cropping resulted in similar or better soybean yield compared to the fallow treatment. A combination of PL and inorganic N produced wheat yields equivalent to or greater than that of inorganic N alone, and the residual PL nutrients in soil slightly enhanced following wheat and soybean grain yields when compared to inorganic N application. Therefore, a combination of PL and inorganic N fertilizer use for a wheat-soybean cropping system could provide sustainable yield production.

2.2 Introduction

Poultry litter (PL) is widely used as an alternative nutrient source to inorganic fertilizer in the southeastern U.S. Application of PL to agricultural fields can increase soil organic matter (Watts

et al., 2010a) and improve soil quality and productivity (Kingery et al., 1994), thereby enhancing crop production (Hirzel et al., 2007a; Mitchell and Tu, 2005; Reddy et al., 2004; Tewolde et al., 2009a; Watts and Torbert, 2011; Wiatrak et al., 2004). However, unlike inorganic N fertilizer, which is 100% available for plants, litter-N is mineralized slowly over time. It is assumed that only 40 to 60% of the PL's total N will become available during the first year of application (Moore et al., 1998; Francesch and Brufau, 2004). The availability of litter-N is also temperature-dependent; thus, potentially changing depending on whether the litter is applied during the winter or summer months. Ruiz Diaz and Sawyer (2008) evaluated the plant availability of N from PL on corn (*Zea mays* L.) production by determining response to grain yield, grain N uptake, and leaf chlorophyll content. They estimated the average first-year plant-available N from PL to be 48% of the total N applied. Gordon et al. (2014) found the potentially available N coefficient for N uptake of winter wheat to be 0.31 when compared to urea ammonium sulfate regardless of PL application timing and the total N rate applied, indicating that more than half of litter N would be available in succeeding years.

Since only a fraction of the N in PL becomes available during the year of application and other PL nutrients accumulate in soil creating a reservoir of nutrients, the residual effects of PL application may maintain subsequent crop yields. The residual effects of PL have been reported in several studies (Nyakatawa et al., 2001; Malik and Reddy, 2002; Hirzel et al., 2007b; Tewolde et al., 2011; Adeli et al., 2015). Nyakatawa et al. (2001) reported that the residual effects of applying PL at 100 kg available N ha^{-1} for 2 years to cotton (*Gossypium hirsutum* L.) under a conservation tillage system increased the following corn grain yield by 13% when compared to the residual effects of inorganic fertilizer. Malik and Reddy (2002) similarly reported that the residual effects of applying PL to cotton for 5 years increased the following corn grain yield

when compared to urea, the year after treatment application had ceased. They reported that applying PL at 40 and 120 kg N ha^{-1} on cotton increased the following corn grain yield by 9 and 81% compared to the control, respectively, while urea at the same N rate increased corn grain yield by 1 and 55% of the control yield, respectively, the year after fertilization ceased (Malik and Reddy, 2002). Adeli et al. (2015) observed increased soybean productivity the first year (no treatment application) following three years of banding pelletized PL to cotton, while no effects were observed the second and third year after treatment application ceased.

Continuous land application of PL based on a crop's N demand can lead to excessive accumulation of soil nutrients (Sharpley et al., 1993; Wood et al., 1996; Bolan et al., 2010), especially P (Chang et al., 1991; Eghball, 2002). This is mainly because the N/P ratio of PL is much lower (about 1:1) than the ratio of N and P (>3:1) removed from soil by crops (Mitchell, 1999). Watts et al. (2010a) reported that long-term application of PL increased Mehlich-1 extractable P by 78% and 175% when compared to inorganic fertilizer plots for soybean and corn cropping systems, respectively. After 4 years of PL application, Sistani et al. (2010a) observed that Mehlich-3 P increased from an initial 31.4 to 63.0 mg kg^{-1} for 4.5 Mg PL ha^{-1} and to 178 mg kg^{-1} for 13.5 Mg PL ha^{-1}. Repeated PL applications may also lead to negative environmental consequences such as heavy metal accumulation, contribution to greenhouse gas emissions, and transport of N and P with surface water runoff (Tewolde et al., 2009b; Sistani et al., 2010b; Watts et al., 2011; Pote and Meisinger, 2014). Therefore, sustainable PL management practices are needed for crop production and environmental protection.

Wheat-soybean double-cropping systems are being widely practiced in the mid-southern U.S., including Alabama (Touchton and Johnson, 1982), Louisiana (Board and Hall, 1984), Arkansas (Caviness et al., 1986), and Mississippi (Hovermale et al., 1992). This practice can

improve fertilizer use efficiency, reduce fertilizer requirements, decrease soil erosion, increase soil organic matter, and reduce soil-water losses from runoff and evaporation (Sanford, 1982; Wesley and Cooke, 1988; Heggenstaller et al., 2008). Caviglia et al. (2011) reported that soybeans under double-cropping systems out-yield those under mono-cropping systems by 58 to 82%, even though the mono-cropped soybeans were sown at the optimum planting date. Nash et al. (2012) applied different N sources (ammonium nitrate, urea, and polymer-coated urea) to wheat at various application dates and rates. They found that wheat yield increased with N rate, but the increases varied across N sources and application dates, while N management (source, rate and application timing) had minimal impact on double-cropped soybean production, including grain yield, oil, and protein concentration in grain. Presently, there is little information about the influence of PL applications on grain yield production from the wheat-soybean double-cropping systems in the southeastern region. The objectives of this research were to (1) compare the response of grain yield on wheat-soybean double cropping and conventional mono-cropping systems; (2) evaluate the impact of inorganic N and PL on wheat and soybean productivity; (3) evaluate the residual effects of PL on wheat and soybean productivity; and (4) investigate the interactions between cropping systems and N fertilizer sources on wheat and soybean production.

2.3 Materials and Methods

2.3.1 Site Description

A two-year field study was initiated in winter of 2014 (2014-2016) and repeated starting in winter of 2015 (2015-2017) at the Alabama Agricultural Experiment Station's E.V. Smith Research Center-Field Crops Unit (EVS) in Macon County, near Shorter, AL (32º25' N, 85º53' W) and Prattville Agricultural Research Unit (PAU) in Autauga County, near Prattville, AL

(32º25' N, 86º26' W). Approximately 77 km separates the two research sites used for this study. The soil was a Marvyn loamy sand at EVS and a Lucedale fine sandy loam at PAU. Climate for both locations is humid subtropical with mean annual precipitation of approximately 1350 mm and a mean annual temperature of 18 ºC (Current Results, 2017). The initial soil properties for each location are presented in Table 1. Before the study initiation in 2014 and 2015, both sites were under mono-cropped soybean production.

2.3.2 Experimental Design and Treatments

The experiments were conducted as a randomized complete block designs with six treatments replicated four times. At each location, the plots were 3.66 by 7.62 m with a 1.22-m buffer separating the plots within each block and a 6.10-m buffer separating the blocks. Fertility treatments for the first year (2014-2015 and repeated in 2015-2016) winter wheat included an unfertilized control (P_0N_0), inorganic N fertilizer at the rate of 135 kg ha^{-1} (P_0N_{135}), PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N ($P_{45}N_{90}$), PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N ($P_{90}N_{45}$), and PL at the rate of 135 kg total N ha^{-1} ($P_{135}N_0$). The properties of the PL used for this study are shown in Table 1. Urea (46% N) was used as the inorganic N fertilizer source. All wheat treatments were double cropped with soybeans (no N fertilizer was added) and compared to a winter wheat fallow treatment. In the second study year (2015-2016 and repeated in 2016-2017), residual effects of PL were tested, so all wheat treatments received urea at the rate of 135 kg N ha^{-1} and soybean received P and K at 45 kg ha^{-1}. An evaluation of grain yield for wheat and soybean was conducted in each study year at each location. Wheat grain yield was not collected at PAU in 2016 for the first year evaluation and at EVS in 2017 for the second year evaluation due to extensive weed pressure.

2.3.3 Cultural Practices

Wheat (AGS 2060 in 2014 and 2015 and AGS 2040 in 2016; Georgia Seed Development, Plains, GA) was sown at a rate of 134 kg ha^{-1} in mid-November of each year for both locations. Poultry litter was applied to the wheat at the time of sowing in 2014 and 2015, and urea was applied to the wheat at the beginning of March each year, except for the P_0N_{135} treatment. Half of the urea was applied at sowing and the other half at the beginning of March fot the P_0N_{135} treatment in the first study year. In the second study year, urea was applied to the wheat at the beginning of March each year. Soybeans (Pioneer 96M60 in 2015 and Pioneer 95M70 in 2016 and 2017; DuPont Pioneer, Johnston, IA) were sown at a rate of 78 kg ha^{-1} in mid-June of each year. No N fertilizer (PL or urea) was applied to the soybean crop. Both PL and urea were surface broadcasted by hand. KCl (0-0-60) and triple super phosphate (0-46-0) were applied to the P_0N_0 and P_0N_{135} treatments at wheat sowing and to all treatments at soybean sowing according to Auburn University's soil testing recommendations. The amount of P and K applied for each treatment for the first study year at both locations is shown in Table 2.

2.3.4 Data Collection

During the growing season, ten plants, randomly chosen within each plot, were used to determine plant height when wheat reached tillering, late stem extension, and the late heading stages, and when soybean reached the full seed (R6) to beginning maturity (R7) stage. Plant height was determined by measuring from the soil surface to the highest growing point of the main stem. Wheat was harvested each year during the beginning of June and soybean during the beginning of November. Wheat and soybean grain yield was determined by mechanically harvesting a 1.5 by 7.6 m long area from the center of each plot using a plot combine. Wheat grain weights were adjusted to a moisture content of 13.5% and soybean grain to a moisture

content of 13%. Precipitation and air temperature data were collected from weather stations located at each experimental site (Fig. 1).

2.3.5 Data Analysis

Wheat and soybean data analyses for the first and second study years were performed separately using the MIXED procedure of SAS 9.4 (SAS Institute Inc., 2013). Cropping systems and fertilization treatments were analyzed as fixed effects, whereas replication, locations, and years were random effects as appropriate. Means were compared using the LSMEANS statement in PROC MIXED and the Tukey's honestly significant difference test at a 0.05 probability level was used to identify significant differences among treatments.

2.4 Results and Discussion

2.4.1 Weather Conditions

Weather conditions varied markedly among the 2-yr of study of 2014-2016 and 2015-2017 (Fig. 1). Average growing season air temperatures were 16.7, 18.1, and 17.9 ºC for 2015, 2016, and 2017 at EVS and 18.5, 20.0, and 19.5 ºC for 2015, 2016, and 2017 at PAU, respectively. Generally, monthly temperatures among growing seasons were normal and did not deviate much (more than 2-3º) from the 30-yr average during the course of this study. Monthly precipitation data collected from EVS showed that totals were 1077 mm in 2015 with 683 and 417 mm occurring during the wheat and soybean growing seasons, 1372 mm in 2016 with 921 and 266 mm occurring during the wheat and soybean growing seasons, and 1218 mm in 2017 with 904 and 253 mm occurring during the wheat and soybean growing seasons, respectively. Monthly precipitation data collected from PAU showed that totals were 1175 mm in 2015 with 699 and 428 mm occurring during the wheat and soybean growing seasons, 1340 mm in 2016 with 977

and 273 mm occurring during the wheat and soybean growing seasons, and 1686 mm in 2017 with 875 and 757 mm occurring during the wheat and soybean growing seasons, respectively.

2.4.2 Effect of PL on Wheat Growth and Grain Yield

Plant height of wheat was greatly affected by the PL and inorganic N treatments relative to the unfertilized control at the various growth stages (Table 3). Combined PL and inorganic N treatments tended to result in taller plants among the growth stages at both locations in 2015 and 2016, especially for the $P_{90}N_{45}$ treatment, which increased plant height up to 81.9% and 45.7% as compared to the control and inorganic N treatment, respectively at the T2 growth stage in 2016 at PAU. The single PL ($P_{135}N_0$) application treatment resulted in taller plants than that of the unfertilized control at the late stem extension (T2, the end of Mar.) and late heading (T3, the end of Apr.) stages, but not at the tillering stage (T1, the end of Feb.) in 2016 and at late stem extension at PAU in 2015. However, $P_{135}N_0$ influence on plant height was not as strong as the other fertilization treatments at the late heading stage (T3), resulting in significantly shorter plants than the inorganic N and the combined PL and inorganic N application treatments (except for at PAU in 2015, which did not differ). This may be due to the slow mineralization of the litter-N not being able to satisfy the high requirement of N needed during the stem elongation stage of wheat. There were no significant differences in plant height of wheat plants among the fertilization treatments during tillering (T1) at the PAU in 2015; however, the high PL application treatments ($P_{90}N_{45}$ and $P_{135}N_0$) produced numerically taller plants, which may be because PL was applied at wheat sowing, so wheat could have obtained N from the PL to support early growth.

Wheat plant height, averaged across treatments and locations, was significantly higher in 2015 (88.7 cm) compared to 57.2 cm for 2016. The plant height difference in 2015 was most

likely due to a higher and more even distribution of rainfall resulting in improved soil moisture availability during the growing season (Fig. 1).

Plant height is an important trait for determining the performance of a wheat crop. In particular, a shorter plant is often associated with an earlier head emergence, leading to a reduction in grain yield (Law et al., 1978), while tall plants are much more susceptible to lodging (Berry et al., 2003). In this study, application of PL plus urea ($P_{90}N_{45}$ and $P_{90}N_{45}$) and urea alone (P_0N_{135}) typically produced taller plants and higher grain yield when compared to the control and the other fertilizer treatments. This suggests that the plant height under the $P_{90}N_{45}$ treatment might be appropriate for wheat productivity, and a similar level of wheat grain production can be achieved with a combination of PL and inorganic N application, compared to conventional inorganic fertilizer application.

There were significant treatment × year and treatment × location interactions for wheat grain yield (Table 4). These interactions reflect the impact of rainfall and soil properties on crop performance. Thus, results are presented and discussed separated by year and location when such interactions occurred.

Wheat grain yield was affected by PL and inorganic N relative to the control in 2015 at EVS and PAU (Table 5). Inorganic N (P_0N_{135}) and treatments with PL combined with inorganic N (i.e., $P_{45}N_{90}$, $P_{90}N_{45}$) had the highest grain yield in 2015 at both locations. For the P_0N_{135} treatment, which was numerically highest, grain yield increased by 135 and 131% over the control at EVS and PAU, respectively. Combining PL and urea produced similar grain yield to that of the single urea application (P_0N_{135}) in 2015 and 2016 at both locations, especially the $P_{90}N_{45}$ treatment which had the highest grain yield in 2015 increasing grain yield by 119 and 111% over the control at EVS and PAU, respectively. Savala et al. (2016) found similar results

in a 2-yr field experiment in North Carolina. They observed higher wheat yield when poultry manure was applied (67 kg N ha^{-1}) with urea ammonium nitrate (UAN, 67 kg N ha^{-1}), while applying PL only had the lowest grain yield when compared to the other fertilizer treatments and was not significantly different from the control. Since nutrient mineralization of manure when applied to soil is microbially-driven, it can be influenced by abiotic factors such as soil temperature, soil moisture, and soil physical properties (Eghball et al., 2002). Watts et al. (2010b) evaluated the seasonal influence of N mineralization from soil amended with composted dairy manure and reported that N mineralization was minimal during winter months. Other researchers have shown that N mineralization decreases with decreasing temperature (Cassman and Munns, 1980; Eghball, 2000; Watts et al., 2007). Therefore, lower yields from the PL only treatments could be related to the slow mineralization capacity of the litter not being able to supply enough plant-available N during the growing season (Rasnake, 2002), thus the wheat's N-requirement was not satisfied.

2.4.3 Residual Effect of PL on Wheat Growth and Grain Yield

Plant height of wheat was greatly affected by the residual influence of PL and inorganic N applications (P_0N_{135}, $P_{45}N_{90}$, $P_{90}N_{45}$, and $P_{135}N_0$) relative to the unfertilized control at the late stem extension (T2) and late heading (T3) stages at EVS and PAU in 2016 and at PAU in 2017 (Table 6). Combined PL and inorganic N, especially the $P_{45}N_{90}$ treatment, tended to result in taller plants at T3 at EVS in 2016 and at T2 at PAU in 2017, which significantly increased plant height by 9.9 and 17.0% as compared to the recommended inorganic fertilizer application (P_0N_{135}), respectively. The PL only ($P_{135}N_0$) application treatment resulted in significantly taller plants than for the unfertilized control among the growth stages (T2 and T3) at both locations in 2016 and at PAU in 2017. This may be due to the mineralization of organic components from

residual PL in soil supplying nutrients for plant growth. Bergstrom and Kirchmann (1999) reported that organic N fractions of PL can release significant amounts of plant-available N, potentially supplying nutrients 2 to 3 years after the initial application. The taller wheat plants observed with PL application treatments ($P_{45}N_{90}$, $P_{90}N_{45}$, and $P_{135}N_0$) indicated that the residual PL in soil could improve plant growth and potentially produce greater crop yield (Nyakatawa et al. 2001; Hirzel et al. 2007b; Tewolde et al. 2011; Ruiz Diaz et al. 2012).

Wheat grain yield was affected by the residual of PL and inorganic N relative to the control in 2016 and 2017 at EVS and PAU (Table 7). All the N fertilization treatments, either urea or PL, produced significantly greater grain yield than the unfertilized control in the second study year. On average, both the PL only ($P_{135}N_0$) and $P_{90}N_{45}$ application had relatively greater grain yield than the urea application only (P_0N_{135}) due to the nutrients being released from the residual PL in soil. Moreover, the $P_{90}N_{45}$ treatment had the highest grain yield in 2016, increasing grain yield by 279 and 48.1% over the control and recommended inorganic N treatment at PAU, respectively. The differences between $P_{90}N_{45}$ and $P_{135}N_0$ treatments may be due to greater crop productivity from the $P_{90}N_{45}$ treatment in the first study year (Tables 5 and 8) leading to more crop residues being left on the soil under no-till conditions (Tewolde et al. 2015). Ruiz Diaz et al. (2012) found similar results in a 3-yr field experiment in Iowa. They observed higher corn yield with PL applications in the second and third years after initial application of PL compared to the unfertilized control and crop yield tended to increase with increasing litter-N rate. The N supply from a single application of solid poultry manure during the second and third years after application has been suggested as supplying 3 to 12% and 0 to 4% of the original total N (Ruiz Diaz et al. 2012). Therefore, greater yields from the applied PL treatments could be related to nutrient mineralization of the residual litter in soil being able to supply extra plant-available

nutrients, especially N, during the growing season (Nyakatawa et al. 2001; Hirzel et al. 2007b; Tewolde et al. 2009b, 2011, 2015).

2.4.4 Soybean Growth and Grain Yield Response to Double-cropping and N Fertilization

Soybean plant height as affected by cropping system and fertilizer are shown for 2015 and 2016 at EVS and PAU in Table 3. Soybean plants under wheat-soybean double cropping were significantly taller than those under the winter fallow treatment in 2015 at PAU, and in 2016 for $P_{45}N_{90}$ and $P_{90}N_{45}$ at EVS, and $P_{135}N_0$ at PAU. Soybean plant height tended to be greater in plots receiving PL, regardless of the rate of PL received, compared to the control plots or plots that received inorganic N for the preceding wheat crop (i.e., 2015 PAU and 2016 EVS). Soybean plants from the single PL application ($P_{135}N_0$) treatments were 16 and 12% taller than the winter fallow control plots at PAU in 2015 and 2016, respectively. The $P_{45}N_{90}$ and $P_{90}N_{45}$ plots were 17 and 22% taller than the winter fallow control plots and 14 and 19% taller than the unfertilized winter wheat control plots at EVS in 2016, respectively. Moreover, the $P_{45}N_{90}$ and $P_{90}N_{45}$ plots also increased soybean plant height by 18 and 15% compared to the winter fallow control at PAU in 2015. Our results were consistent to those observed by Adeli et al. (2015), which reported that soybean plants grown in plots that received pelletized broiler litter the preceding 3 years were significantly taller than the control or inorganic N plots. This study also suggests that adding poultry litter to a winter crop may also positively benefit the succeeding summer crop of a double cropping system.

Statistical analysis for soybean grain yield indicated that there were significant treatment × year and treatment × year × location interactions (Table 4). These interactions reflected the impact of climatic conditions and soil properties on crop performance. As a result, data are presented and discussed separately by year and location when such interactions occurred.

Overall soybean grain yield was greater in 2015 (1559.7 kg/ha) than 2016 (1009.9 kg/ha) (Table 6). In 2015, rainfall was higher and more evenly distributed during the growing season (Fig. 1). In 2016, yield at EVS was considerably lower than average. Overall, soybean grain yield was greater at PAU (1817.9 kg/ha) than EVS (751.7 kg/ha). The grain yield difference at PAU is most likely due to soil texture (Table 1), which has a higher water and nutrient holding capacity, and may have reduced the impact of lower and irregular rainfall in 2015.

Soybean grain yield was 71 to 95% greater in the wheat-soybean double cropping compared to the winter fallow-soybean mono-cropping system in 2015 at EVS, while no difference was observed in 2016 at EVS or in either year at PAU (Table 6). Caviglia et al. (2011) reported that there was no yield difference between mono- and double-cropped soybean productivity if the seed was sown on the same date. A long-term field study found that the soybean grain yield varied through the years with mono-cropped soybeans producing higher yields compared to no-till double-cropped soybeans in 6 of 11 years, and disparities were attributed to differences in rainfall and temperature during the soybean growing seasons (Crabtree et al., 1990). Results from Crabtree et al. (1990) suggest that double cropping could increase soybean productivity under appropriate weather conditions (adequate rainfall). This increase in soybean productivity may be due to the previous wheat crop protecting the soil during winter months and the residual wheat nutrients enhancing the soil nutrient levels for soybean growth.

No significant soybean grain yield differences were observed among N sources (Table 6). Watts and Torbert (2011) reported that soybean grain yield increased 8 out of 9 yrs when poultry litter was applied to a fine sandy loam (Appalachian Plateau region). When soybean was planted in a loam soil (Blackland Prairie region) following 3-yr pelletized broiler litter treated cotton, soybean productivity increased the first year after the last application, but not the following year

2 or 3 when compared to residual UAN application (Adeli et al., 2015). This study shows that residual effects of applying a single application of PL to winter wheat may not increase the double-cropped soybean grain yield on a Coastal Plain soil (less productive than the Appalachian Plateau and Blackland Priarie soils; Adeli et al., 2015).

2.5 Conclusion

Compared to inorganic fertilizer, a single application of PL was less effective on winter wheat yield, and this is likely due to the litter's slow N mineralization capacity. While a combination of PL and inorganic N produced wheat yields equivalent to or greater than that of inorganic N application, and the residual nutrients from PL additions were slightly enhanced following wheat and soybean productivity. These results indicate that a combination of PL and inorganic N is a good alternative to chemical fertilizer for the growth and productivity of wheat and soybean. Wheat-soybean double cropping improved soybean growth and yield compared to the fallow-soybean mono-cropping system, which may be due to the wheat protecting the soil during winter months and the residual nutrients from the winter wheat crop enhancing soil productivity for soybean growth. Therefore, a combination of PL and inorganic N fertilizer use for a wheat-soybean cropping system could provide sustainable yield production.

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Table 1. Initial soil properties for EVS and PAU and poultry litter properties on a dry weight basis in 2014 and 2015.

Property†	EVS soil		PAU soil		Poultry litter	
	2014-2016	2015-2017	2014-2016	2015-2017	2014-2016	2015-2017
pH (1:1 soil:water)	5.8	6.5	6.3	6.3	-	-
Moisture content (g 100 g^{-1})	-	-	-	-	17.9	18.5
Total C (g kg^{-1})	3.5	3.8	9.9	8.9	269.5	284
Total N (g kg^{-1})	0.30	0.50	0.90	1.00	37.8	35.8
C:N ratio	11.7	7.6	11	8.9	7.13	7.93
P (g kg^{-1})	0.02	0.02	0.02	0.03	19.4	19.7
K (g kg^{-1})	0.05	0.06	0.15	0.21	37.6	36.8
Ca (g kg^{-1})	0.20	0.21	0.92	0.66	41.1	34.7
Mg (g kg^{-1})	0.05	0.07	0.11	0.10	9.4	9.7
Na (g kg^{-1})	0.03	0.03	0.04	0.03	16.23	15.54
Cu (mg kg^{-1})	2.18	1.36	1.46	0.99	565	716
Fe (mg kg^{-1})	10.5	7.9	8.0	11.7	2222	4132
Mn (mg kg^{-1})	9.9	7.8	61.0	59.8	634	655
Zn (mg kg^{-1})	1.9	2.9	2.9	1.9	578	690

†P, K, Ca, Mg, Na, Cu, Fe, Mn, and Zn values represent Mehlich-1 extractable nutrient concentrations for soil and total nutrient concentrations for poultry litter.

Table 2. The application rate of nitrogen (N), phosphorus (P), and potassium (K) to wheat and soybean for each treatment in 2014-2015 and 2015-2016 for EVS and PAU.

Treatment†	Wheat			Soybean		
	N	P	K	N	P	K
	kg ha^{-1}					
Fallow	-	-	-	-	45	45
P_0N_0	0	45	45	-	45	45
P_0N_{135}	135	45	45	-	45	45
$P_{45}N_{90}$	135	23, 25‡	45, 46	-	45	45
$P_{90}N_{45}$	135	46, 50	90, 93	-	0	0
$P_{135}N_0$	135	69, 78	134, 139	-	0	0

†P_0N_0-unfertilized control; P_0N_{135}-inorganic N fertilizer at the rate of 135 kg ha^{-1}; $P_{45}N_{90}$-PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N; $P_{90}N_{45}$-PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N; and $P_{135}N_0$-PL at the rate of 135 kg total N ha^{-1}.

‡The two values are for the 2014-2015 and 2015-2016 study years, respectively, due to the different nutrient properties of PL used in these two years.

Table 3. Response of plant height (cm) measured at the various growth stages for cropping system and fertilizer sources at EVS and PAU in 2015 and 2016 (first study year).

Site	Treatment†	Wheat					Soybean	
		2015			2016		2015	2016
		T1	T2	T3	T2	T3		
EVS	Fallow	-	-	-	-	-	50.0 ± 1.21	36.9 ± 0.73 c
	P_0N_0	11.3 ± 0.44 ab‡	46.6 ± 1.40	83.5 ± 1.53 b	40.6 ± 1.39 c	52.2 ± 1.12 d	50.1 ± 1.20	37.9 ± 0.80 c
	P_0N_{135}	10.3 ± 0.44 c	50.3 ± 1.32	90.4 ± 1.25 a	44.7 ± 1.44 bc	62.2 ± 1.09 b	50.4 ± 1.08	39.9 ± 0.95 bc
	$P_{45}N_{90}$	9.26 ± 0.61 c	48.4 ± 1.42	87.9 ± 1.17 ab	47.5 ± 1.27 b	63.7 ± 0.97 b	46.9 ± 1.15	43.1 ± 1.41 ab
	$P_{90}N_{45}$	12.2 ± 0.72 ab	50.9 ± 1.17	91.2 ± 1.29 a	54.6 ± 1.24 a	68.4 ± 0.87 a	46.3 ± 1.19	45.1 ± 1.27 a
	$P_{135}N_0$	12.4 ± 0.44 a	47.1 ± 1.42	83.9 ± 1.40 b	47.1 ± 1.53 b	56.7 ± 1.16 c	50.0 ± 1.19	40.0 ± 0.68 bc
PAU	Fallow	-	-	-	-	-	59.0 ± 0.88 B	63.9 ± 1.88 B
	P_0N_0	13.2 ± 0.43	46.8 ± 1.56 B	84.5 ± 1.20 B	24.9 ± 1.18 D	39.3 ± 0.98 C	66.7 ± 1.00 A	69.0 ± 1.14 AB
	P_0N_{135}	13.4 ± 0.63	51.8 ± 0.92 A	92.6 ± 0.92 A	31.1 ± 0.84 C	56.5 ± 2.16 AB	66.6 ± 1.10 A	70.4 ± 2.57 AB
	$P_{45}N_{90}$	13.0 ± 0.45	51.9 ± 1.08 A	91.7 ± 0.77 A	39.1 ± 1.21 B	59.5 ± 1.07 A	69.4 ± 1.20 A	67.6 ± 1.74 AB
	$P_{90}N_{45}$	14.5 ± 0.59	54.6 ± 1.05 A	91.5 ± 2.32 A	45.3 ± 1.31 A	60.8 ± 0.92 A	68.0 ± 1.09 A	68.3 ± 1.67 AB
	$P_{135}N_0$	14.4 ± 0.62	52.1 ± 1.55 A	89.4 ± 1.25 AB	46.5 ± 1.49 A	52.9 ± 1.52 B	68.2 ± 0.90 A	71.3 ± 2.18 A
		P > F (0.05)						
	EVS	<0.0001	0.0583	<0.0001	<0.0001	<0.0001	0.0314	<0.0001
	PAU	0.0944	0.0004	0.0002	<0.0001	<0.0001	<0.0001	0.0482

†P_0N_0-unfertilized control; P_0N_{135}-inorganic N fertilizer at the rate of 135 kg ha^{-1}; $P_{45}N_{90}$-PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N; $P_{90}N_{45}$-PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N; and $P_{135}N_0$-PL at the rate of 135 kg total N ha^{-1}. T1, tillering stage; T2, late stem extension stage; T3, late heading stages. For 2016, the wheat height at T1 was too small to measure, thus, no data presented at this stage.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$. Small letters show multiple comparisons of treatments at EVS, and capital letters show multiple comparisons of treatments at PAU.

Table 4. Analysis of variance results for wheat and soybean grain yield for the first and second study years at EVS and PAU.

Source	P > F (0.05)			
	1st year		2nd year	
	Wheat	Soybean	Wheat	Soybean
Treatments (T)	<0.0001	0.0161	< 0.0001	< 0.0001
Year (Y)	<0.0001	<0.0001	0.0041	0.0002
Location (L)	<0.0001	<0.0001	0.4931	< 0.0001
T × Y	0.0013	0.0138	0.2443	0.4104
T × L	0.0313	0.6744	0.0504	0.0755
T × Y × L	-	< 0.0001	-	0.0005

Table 5. Effect of poultry litter and urea application on wheat grain yield (kg ha^{-1}) for first study year (2015 and 2016) at EVS and PAU.

Treatment†	EVS		PAU	Mean
	2015	2016	2015	
P_0N_0	1419 ± 203.8 b‡	682.6 ± 157.0	2174 ± 249.8 b	1425 ± 213.1
P_0N_{135}	3332 ± 204.6 a	1110 ± 222.2	5027 ± 292.2 a	3156 ± 500.0
$P_{45}N_{90}$	2659 ± 442.3 a	1139 ± 166.4	4569 ± 92.59 a	2789 ± 447.3
$P_{90}N_{45}$	3113 ± 338.4 a	1483 ± 295.6	4592 ± 196.7 a	3063 ± 410.4
$P_{135}N_0$	1692 ± 98.17 b	777.9 ± 139.3	3005 ± 326.7 b	1825 ± 297.2
	P > F (0.05)			
	< 0.0001	0.1195	<0.0001	-

†P_0N_0-unfertilized control; P_0N_{135}-inorganic N fertilizer at the rate of 135 kg ha^{-1}; $P_{45}N_{90}$-PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N; $P_{90}N_{45}$-PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N; and $P_{135}N_0$-PL at the rate of 135 kg total N ha^{-1}. No wheat yield data are presented for PAU in 2016 due to extensive weed pressure.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

Table 6. Response of plant height (cm) measured at the various growth stages for cropping system and fertilizer sources at EVS and PAU in 2016 and 2017 (second study year).

Site	Treatment†	Wheat					Soybean	
		2016		2017			2016	2017
		T2	T3	T1	T2	T3		
EVS	Fallow	-	-	-	-	-	44.4 ± 0.93 a	45.9 ± 1.48 bc
	P_0N_0	33.5 ± 0.89 b‡	58.1 ± 0.95 c	10.0 ± 0.54	29.2 ± 0.73	-	41.6 ± 0.75 ab	41.6 ± 1.15 c
	P_0N_{135}	38.5 ± 0.86 a	64.7 ± 1.39 b	10.7 ± 0.54	29.4 ± 0.93	-	43.5 ± 0.87 a	45.8 ± 1.28 bc
	$P_{45}N_{90}$	38.3 ± 0.91 a	71.1 ± 1.31 a	10.7 ± 0.54	29.2 ± 1.19	-	41.4 ± 0.69 ab	47.6 ± 1.43 ab
	$P_{90}N_{45}$	37.7 ± 0.86 a	65.4 ± 1.30 b	10.1 ± 0.40	28.5 ± 0.93	-	39.9 ± 0.93 b	50.9 ± 1.22 a
	$P_{135}N_0$	38.7 ± 0.72 a	67.3 ± 1.35 ab	11.0 ± 0.52	31.6 ± 0.91	-	43.4 ± 0.63 a	48.6 ± 1.56 ab
PAU	Fallow	-	-	-	-	-	59.1 ± 1.14 d	57.4 ± 1.76 c
	P_0N_0	59.2 ± 2.01 c	63.5 ± 1.57 b	7.08 ± 0.37	18.5 ± 0.74 c	60.7 ± 1.53 b	71.1 ± 1.45 c	57.7 ± 1.26 c
	P_0N_{135}	68.3 ± 1.26 a	73.8 ± 1.24 a	6.34 ± 0.39	27.6 ± 1.25 b	73.3 ± 1.53 a	83.1 ± 1.03 ab	72.3 ± 1.70 ab
	$P_{45}N_{90}$	63.4 ± 1.02 bc	76.3 ± 1.19 a	6.63 ± 0.35	32.3 ± 1.23 a	74.2 ± 2.20 a	81.5 ± 2.14 b	70.0 ± 2.13 b
	$P_{90}N_{45}$	67.7 ± 1.26 ab	75.2 ± 1.44 a	6.49 ± 0.35	29.1 ± 1.14 ab	72.7 ± 1.33 a	83.3 ± 1.06 ab	69.6 ± 1.40 b
	$P_{135}N_0$	69.0 ± 1.45 a	76.5 ± 1.27 a	7.09 ± 0.36	28.4 ± 1.23 ab	74.4 ± 1.20 a	88.3 ± 1.31 a	77.7 ± 1.26 a
		P > F (0.05)						
	EVS	< 0.0001	< 0.0001	0.5903	0.1353	-	0.0007	< 0.0001
	PAU	< 0.0001	< 0.0001	0.4695	< 0.0001	< 0.0001	< 0.0001	< 0.0001

†P_0N_0-unfertilized control; P_0N_{135}-inorganic N fertilizer at the rate of 135 kg ha^{-1}; $P_{45}N_{90}$-PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N; $P_{90}N_{45}$-PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N; and $P_{135}N_0$-PL at the rate of 135 kg total N ha^{-1}. T1, tillering stage; T2, late stem extension stage; T3, late heading stages. For 2016, the wheat height at T1 was too small to measure, thus, no data presented at this stage, and for 2017, the wheat height at EVS was not measured due to the extensive weed pressure.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$. Small letters show multiple comparisons of treatments at EVS, and capital letters show multiple comparisons of treatments at PAU.

Table 7. Residual effect of poultry litter and urea application on wheat grain yield (kg ha^{-1}) for the second study year (2016 and 2017) at EVS and PAU.

Treatment†	EVS	PAU		Mean
	2016	2016	2017	
P_0N_0	349.2 ± 16.7 b‡	319.7 ± 36.8 c	507.8 ± 28.8 b	392.2 ± 29.1
P_0N_{135}	965.1 ± 67.6 a	818.2 ± 96.1 b	1020 ± 45.2 a	934.5 ± 45.9
$P_{45}N_{90}$	941.3 ± 26.2 a	869.3 ± 69.2 b	1049 ± 121 a	961.0 ± 49.5
$P_{90}N_{45}$	994.9 ± 56.2 a	1212 ± 39.8 a	1158 ± 149 a	1113 ± 60.9
$P_{135}N_0$	1071 ± 49.2 a	956.3 ± 27.5 b	1051 ± 107 a	1032 ± 42.1
	P > F (0.05)			
	< 0.0001	< 0.0001	0.0028	-

†P_0N_0-unfertilized control; P_0N_{135}-inorganic N fertilizer at the rate of 135 kg ha^{-1}; $P_{45}N_{90}$-PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N; $P_{90}N_{45}$-PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N; and $P_{135}N_0$-PL at the rate of 135 kg total N ha^{-1}. No wheat yield data are presented for EVS in 2017 due to extensive weed pressure.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

Table 8. Effect of poultry litter and urea application, and cropping system on soybean grain yield (kg ha^{-1}) for the first study year (2015 and 2016) at EVS and PAU.

Treatment†	EVS		PAU		Mean
	2015	2016	2015	2016	
Fallow	738.4 ± 115.9 b‡	374.4 ± 115.6	1706 ± 40.84	1724 ± 126.2	1136 ± 160.3
P_0N_0	1274 ± 95.90 a	182.6 ± 51.5	1919 ± 27.09	1639 ± 52.37	1302.5 ± 178.2
P_0N_{135}	1437 ± 124.0 a	348.1 ± 92.77	1887 ± 101.1	1542 ± 232.5	1402 ± 166.9
$P_{45}N_{90}$	1345 ± 131.5 a	179.5 ± 63.18	1930 ± 71.72	1873 ± 377.6	1375.3 ± 201.8
$P_{90}N_{45}$	1265 ± 94.35 a	414.4 ± 237.5	2047 ± 71.41	1806 ± 184.0	1447.8 ± 171.8
$P_{135}N_0$	1289 ± 40.63 a	172.8 ± 39.50	1879 ± 160.8	1863 ± 163.7	1341.3 ± 190.2
	P > F (0.05)				
	0.0013	0.5328	0.1329	0.8749	-

†P_0N_0-unfertilized control; P_0N_{135}-inorganic N fertilizer at the rate of 135 kg ha^{-1}; $P_{45}N_{90}$-PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N; $P_{90}N_{45}$-PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N; and $P_{135}N_0$-PL at the rate of 135 kg total N ha^{-1}.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

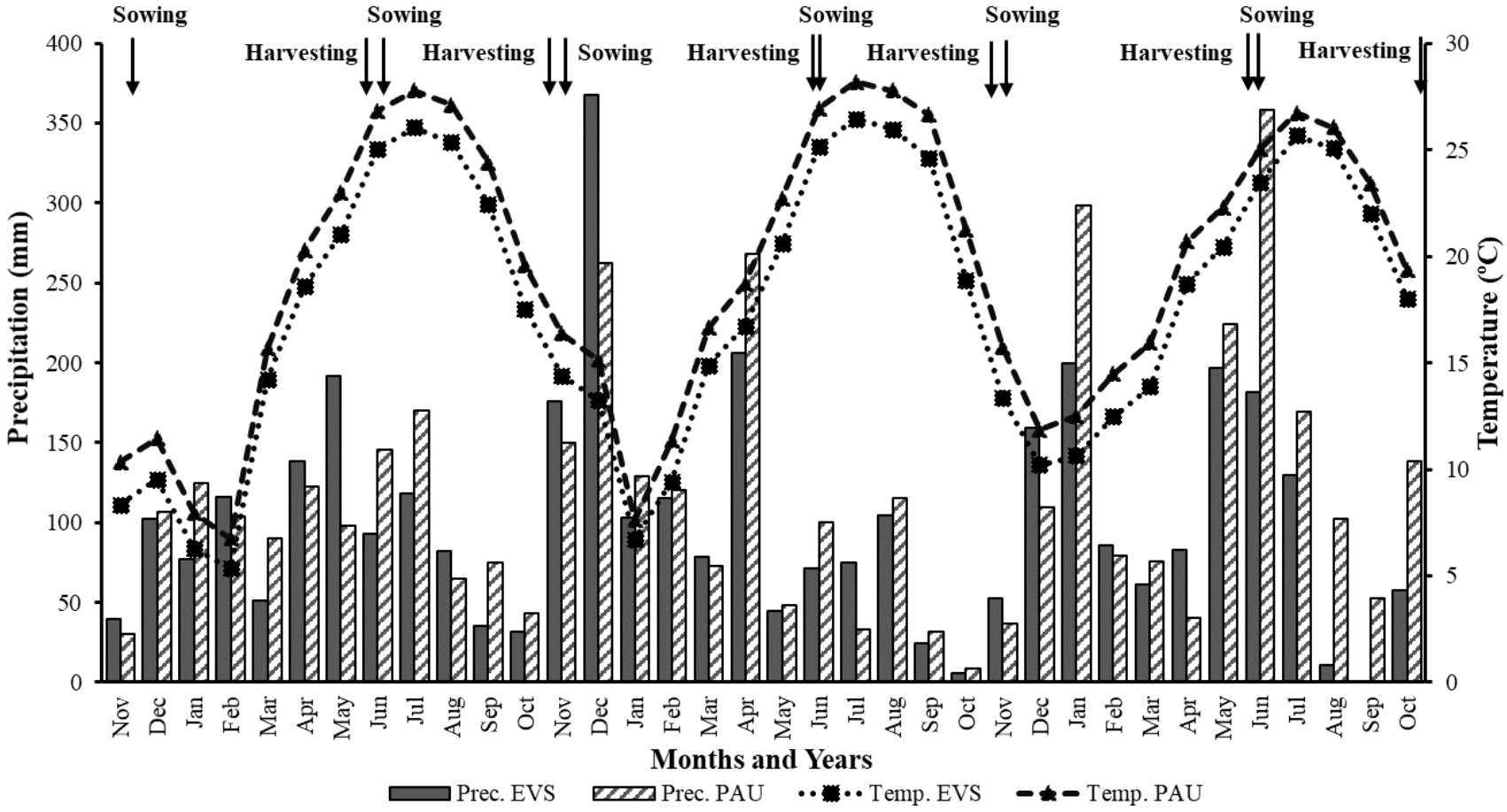
Table 9. Effect of poultry litter and urea application, and cropping system on soybean grain yield (kg ha^{-1}) for the second study year (2016 and 2017) at EVS and PAU.

Treatment†	EVS		PAU		Mean
	2016	2017	2016	2017	
Fallow	1057 ± 91.6 b‡	1089 ± 55.5 b	1392 ± 189 b	2002 ± 119 b	1385 ± 113
P_0N_0	1341 ± 117 ab	1265 ± 92.1 b	1946 ± 164 ab	2129 ± 208 b	1670 ± 118
P_0N_{135}	1725 ± 153 a	1509 ± 172 b	2172 ± 146 a	2957 ± 92.0 a	2033 ± 155
$P_{45}N_{90}$	1485 ± 53.4 ab	1712 ± 75.2 ab	2118 ± 196 ab	2482 ± 175 ab	1949 ± 117
$P_{90}N_{45}$	2040 ± 86.1 a	2057 ± 171 a	2446 ± 306 a	2958 ± 72.2 a	2336 ± 128
$P_{135}N_0$	1884 ± 199 a	1663 ± 161 ab	1996 ± 73.1 ab	2287 ± 116 ab	1958 ± 87.1
	P > F (0.05)				
	0.0012	0.0004	0.0110	0.0058	-

†P_0N_0-unfertilized control; P_0N_{135}-inorganic N fertilizer at the rate of 135 kg ha^{-1}; $P_{45}N_{90}$-PL at the rate of 45 kg total N ha^{-1} plus 90 kg ha^{-1} inorganic N; $P_{90}N_{45}$-PL at the rate of 90 kg total N ha^{-1} plus 45 kg ha^{-1} inorganic N; and $P_{135}N_0$-PL at the rate of 135 kg total N ha^{-1}.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

Fig. 1. Monthly average air temperature and precipitation totals at the Alabama Agricultural Experiment Station's E. V. Smith Research Center (EVS) and Prattville Agricultural Research Unit (PAU) for November 2014 to October 2017.



3. Effect of Nitrogen Fertilization on Winter Canola Yield and Nitrogen Uptake

3.1 Abstract

Canola (*Brassica napins* (L.)) has the potential for being used in a double-cropping system as a winter crop in the southeastern US, but little information is known about its fertility requirements when grown in this region. Increasing fertilizer costs have also resulted in the interest of using poultry litter (PL) as an alternative nutrient source for crops in the Southeast. However, the effectiveness of using PL as a fertilizer source for winter crops is not well understood. Thus, two field studies were conducted to (1) evaluate the optimal N rate for canola production; (2) evaluate yield response of canola to PL application when compared to inorganic N fertilizer at two locations (Shorter, AL - Compass loamy sand; and Prattville, AL - Lucedale fine sandy loam). The experimental design was a randomized complete block with four replications. The N rates included were 0, 68, 135, 180, 202, and 270 kg N ha^{-1}. In the second study, fertility treatments consisted of an unfertilized control, inorganic N fertilizer (urea, 180 kg N ha^{-1}), PL at 68 kg N ha^{-1} plus 112 kg N ha^{-1} urea, PL at 112 kg N ha^{-1} plus 68 kg N ha^{-1} urea, and PL at 180 kg N ha^{-1}. Canola growth, yield, and N uptake are highly dependent on N fertility. The optimal N rate observed in this study was 197 to 232 kg N ha^{-1} for these southeastern US soils. Combining PL and urea significantly increased canola growth and provided greater seed yield compared to a single PL application or the control. The PL at 68 kg N ha^{-1} plus 112 kg N ha^{-1} urea treatment resulted in an equivalent or slightly greater aboveground biomass, seed yield, and N uptake compared to the recommended urea treatment. Therefore, a combination of PL and inorganic N fertilizer could provide sustainable canola yield production.

3.2 Introduction

Canola production has increased rapidly over the past two decades, rising to the second largest oil crop in the United States with more than 2.16 million acres being planted in 2017 (USDA-NASS, 2017). Most of US canola production is concentrated in the Northwest, where more than 80% of the total production occurs (USDA-NASS, 2017). Due to high consumption requirements and increased profits, winter canola acreage has been expanding into southern states, such as Oklahoma, Alabama, South Carolina, Florida, and Georgia (Buntin et al. 2010; USDA-NASS, 2017).

Mild winters with adequate rainfall, availability of local soybean oil processing facilities, and potential for double cropping makes the southeastern US a promising region for canola production. Interest in using canola as an alternative winter crop for wheat (*Triticum aestivum* L.), has increased in the southeastern region. Raymer et al. (1990) indicated that winter canola could not only be used to reduce insect damage, break disease cycles, and decrease weed pressure that may occur with wheat, but it could also be a more profitable crop than wheat. In addition, rotating winter canola with winter wheat, instead of continuously growing winter wheat, can improve marketability of the wheat because of improved consistency and quality after a canola rotation (Boyles et al. 2004). Bishnoi et al. (2007) set up a series of field experiments to evaluate the agronomic performances of winter canola in the southeastern US and found that canola planted in early October with a seeding rate of 6.0 kg ha^{-1} and receiving 180 kg N ha^{-1} gave the highest seed yield in this region. Similarly, Zheljazkov et al. (2013) showed that increasing N rates (0 to 180 kg N ha^{-1}) resulted in greater seed yield and oil content, and suggested that winter canola could be successfully planted in hot humid environments of the southeastern US and produce seed and oil yields comparable to those in major canola production areas. However, canola research in the southeastern region has been limited and most fertility

studies to date have focused on seed and oil yield response to chemical fertilizer applications (Raymer et al. 1990; Porter 1993; Usherwood 1993; Bishnoi et al. 2007; Zheljazkov et al. 2013). In addition, over application of inorganic fertilizer not only increases input costs, but may lead to environmental problems, such as water pollution and soil degradation (Bennett et al. 2001; Baumhardt et al. 2015).

Poultry litter is a good source of organic nutrients, containing both macro- and micronutrients, and its application can increase soil organic matter (Watts et al., 2010), thereby improving soil quality and productivity (Kingery et al., 1994). Numerous studies have shown that PL is an effective alternative to inorganic fertilizer and may produce yields equivalent to or greater than those of IF sources (Reddy et al. 2004; Wiatrak et al. 2004; Mitchell and Tu 2005; Hirzel et al. 2007; Tewolde et al. 2009; Tewolde et al. 2010; Watts and Torbert 2011). For instance, Watts and Torbert (2011) reported that the yield of soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays* L.) was increased 8 out of 9 years and 3 out of 9 years, respectively with PL application when compared to the inorganic fertilizer treatment. Tewolde et al. (2010) indicated that cotton (*Gossypium hirsutum* L.) fertilized with broiler litter had lower tissue N concentration, chlorophyll index, and comparable leaf area index, but produced more lint yield than that with ammonium nitrate. However, the use of PL alone may not meet all of the plant nutrient requirements (primarily N) due to slow mineralization of organic forms of nutrients (nutrient mineralization rate is temperature-dependent), especially during winter months. In reviewing 90 independent studies, Lin et al. (2017) reported that PL may not always increase crop yield when compared to inorganic fertilizer during first the year of application, while combining PL and inorganic fertilizer resulted in an 18% increase in crop yield when compared to inorganic fertilizer application alone. According to Adeli et al. (2007), application of 4.5 Mg

ha^{-1} PL supplemented with 67 kg N ha^{-1} inorganic N increased cotton lint yield as compared to PL at 6.7 Mg ha^{-1} and inorganic fertilizer (urea-ammonium nitrate at recommended N rate) alone during a three-year field study. Another field study conducted in the southeastern US showed that a single PL application did not improve either wheat or soybean production, while adding both PL and inorganic N increased winter wheat yield and the residual effects enhanced double cropped soybean productivity (Lin et al. 2017). Increased grain yield, dry-matter production, and nutrient uptake was also observed with corn when the application of PL and mineral N and P fertilizer were combined (Fallah et al. 2013). Integration of PL and inorganic N may be an efficient nutrient management practice for crop productivity instead of applying PL or inorganic fertilizer alone.

Several studies have been conducted to evaluate the response of canola on cattle manure (Hao et al. 2004; Lupwayi et al. 2014), hog manure (Lafond 2004; Katanda et al. 2016), and swine manure (Qian and Schoenau 2000). For instance, Stevenson et al. (1998) reported that application of cattle manure had 22% lower grain yield and slightly lower grain N concentration and residue (straw and chaff) yield for canola in a three-year field study. In contrast, the use of broiler litter at the rate of 9.0 or 13.5 Mg ha^{-1} as a nutrient source resulted increased grain yield of canola under a double-cropping system (Gascho et al. 2001). However, little research has focused on PL or the integration of PL with mineral fertilizer on canola productivity. As the demand for eatable oil and biodiesel from canola continues to expand, balances between fertilizer applications and sustaining the environment, increasing grain yield of canola with optimal field management has become an urgent issue for farmers and researchers. Thus, the objectives of this study were to: (1) evaluate the optimal N rate for canola production, (2)

evaluate the effects of PL and urea applications on canola growth and grain yield, and (3) determine the N fertilizer management for canola production in the southeastern region.

3.3 Materials and Methods

3.3.1 Site Description

A field study was initiated in winter of 2016 at the Alabama Agricultural Experiment Station's E.V. Smith Research Center-Field Crops Unit (EVS) in Macon County, near Shorter, AL (32º26'N, 85º52'W) and Prattville Agricultural Research Unit (PAU) in Autauga County, near Prattville, AL (32º25'N, 86º26'W). Approximately 77 km separates the two research sites used for this study. Climate for both regions is humid subtropical with mean annual precipitation of approximately 1350 mm and a mean annual temperature of 18 ºC (Current Results, 2017). The soil was a Compass loamy sand (coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults) at EVS and a Lucedale fine sandy loam (fine-loamy, siliceous, subactive, thermic Rhodic Paleudult) at PAU. Both soil series are from soil types typically found in the Southern Coastal Plain region. The Compass series consists of very deep, moderately well drained, moderately slowly permeable soil on the broad uplands and sloping side slopes that lead to drainageways. The Lucedale series consists of deep, well drained, moderately permeable soils that form in loamy sediments, ranging from nearly level to strongly sloping. The initial soil properties are shown in Table 1. Before the study was initiation in 2016, both sites had been under intensive row crop production.

3.3.2 Experiment Setup

Experiment 1: Nitrogen rate study

The experimental design was a randomized complete block with six treatments and four replicates blocked based on slope. Treatments for the winter canola crop consisted of six

incremental N rates: urea (46% N) applied 68, 135, 180, 202, and 270 kg N ha^{-1}, an unfertilized control; 180 kg N ha^{-1} is the recommended N rate according Auburn University soil testing recommendations.

Both research sites were prepared by disking, field cultivating, and then rotovating. The plots were 3.66 by 7.62 m long with a 1.22-m buffer separating the plots in each block and a 6.10-m buffer separating the blocks.

Canola (Inspiration; Rubisco Seeds LLC., Philpot, KY) was sown to a depth of 0.64 cm at a rate of 5.6 kg ha^{-1} in late October, 2016 at both EVS and PAU using a no-till drill (Great Plains 1205NT Drill, Great Plains Manufacturing, Salina, KS, USA) with a 19 cm row spacing. At sowing, 40 kg ha^{-1} triple super phosphate (0-46-0) and KCl (0-0-60) were applied to all treatments based according Auburn University soil testing recommendations. Flue gas desulfurization (FGD) gypsum ($CaSO_4$; 18% S), was as applied as the sulfur source in split applications to all treatments; half of the gypsum (20 kg ha^{-1}) applied at canola sowing and the other half applied on 01 March, 2017. Boron was applied at 1.2 kg B ha^{-1} at the beginning of flowering stage. Urea was also applied in split applications; at sowing, 01 March, and 01 April (Table 2, Buntin et al. 2010). All fertilizers were surface broadcasted by hand. Weeds were controlled with herbicides as needed according to Alabama Cooperative Extension System's recommendations.

Experiment 2: Integration of PL and inorganic N study

The experiment was conducted as a randomized complete block design with five treatments and four replicate blocks laid out based on landscape position. Prior to laying out experimental plots at both locations, the entire site area was prepared by disking, field cultivating and rotovating. Each plot was 3.66 by 7.62 m with a 1.22-m buffer separating the plots in each block

and a 6.10-m buffer separating the blocks. Fertility treatments for winter canola included an unfertilized control (P_0U_0), urea (46% N) as inorganic N fertilizer (180 kg ha^{-1}, P_0U_{180}), PL at a rate of 68 kg total N ha^{-1} plus 112 kg ha^{-1} inorganic N ($P_{68}U_{112}$), PL at a rate of 112 kg total N ha^{-1} plus 68 kg ha^{-1} inorganic N ($P_{112}U_{68}$), and PL at a rate of 180 kg total N ha^{-1} ($P_{180}U_0$). The properties of the PL used in this study are shown in Table 1.

Poultry litter was applied to the canola at sowing, and urea was applied in split applications to the canola at different growth stages: For the P_0U_{180} treatment, 45 kg N ha^{-1} urea was applied at sowing, 45 kg N ha^{-1} urea applied on 01 March, 2017, and the remaining 90 kg N ha^{-1} urea applied on 01 April, 2017. For the $P_{68}U_{112}$ treatment, half of the urea was applied on 01 March and the other half on 01 April, 2017; For the $P_{112}U_{68}$ treatment, urea was applied on 01 April, 2017.

3.3.3 Measurements

Canola growth measurements were taken during the growing season. Ten plants randomly chosen within each plot were used to determine plant height and leaf greenness (SPAD readings) before urea application (01 March and 01 April, 2017). At the beginning of the seed ripening stage, another ten plants were randomly collected within each plot to determine plant height, leaf greenness, biomass and N content. Plant height was determined by measuring from the soil surface to highest growing point of the main stem. Leaf greenness was determined using a Minolta SPAD meter (Minolta Co., Ltd., Osaka, Japan). SPAD readings were conducted on the uppermost fully developed canola leaf's adaxial side. To determine total aboveground biomass, the plants were cut with pruning clippers 5 cm above the soil surface (1 m border from the edge of the plot) and total fresh weight was recorded in the field using a digital hanging scale. The plants were then coarsely ground using a Cub Cadet Chipper (Cub Cadet, Cleveland, OH, USA).

Afterwards, subsamples of the ground plant material were collected, placed in cloth bags, and brought back to the laboratory for moisture determination. The subsamples were dried in a forced-air drying oven at 55 ºC until weight became constant (approximately 5 days). Once more than 50% of the silique turned brown (in the beginning of June), canola leaves were desiccated using Gramoxone. A week later, canola grain yield was determined by mechanically combining the entire length of the center in each plot using an ALMACO SPC 40 plot combine (ALMACO, Nevada, Iowa, USA) at EVS and a Massey Ferguson 8XP plot combine (Massey Ferguson, Duluth, GA, USA) at PAU, and both had a 127 cm header width. The harvested grain reported for this study was adjusted to 10% moisture content. Analysis for total N was performed on the plant tissue and seed samples. The samples were ground to pass through a 0.2 mm mesh sieve prior to nutrient analysis. Total N was determined by the combustion method using a LECO FP-528 Nitrogen/Protein Analyzer (LECO Corp., Saint Joseph, MI, USA).

3.3.4 Data Analysis

Experiment 1: Nitrogen rate study

Data collected were analyzed using an ANOVA and regression model with the R statistical language, version 3.3.3 (https://www.r-project.org/). The Tukey's honestly significant difference test at a 0.05 probability level was used to identify significant differences among treatments. Linear plateau and exponential models were used to fit seed yield and N uptake response lines for the applied N. The linear-plateau model is defined by

$$Y = a + bX \text{ for } X < C \qquad [1]$$

$$Y = P \qquad \text{for } X \geq C \qquad [2]$$

where Y is the yield of grain (kg ha^{-1}), X is the rate of N application (kg ha^{-1}), a is the intercept parameter, b is the slope parameter, and C is the critical N rate, which occurs at the intersection

of the linear response and the plateau line, and P is the plateau or maximum yield obtained by fitting the model to the data. The exponential model is defined by

$$Y = M(1 - \exp^{-a(X+b)}) \qquad [3]$$

where Y is the yield of grain (kg ha^{-1}), X is the rate of N application (kg ha^{-1}), a refers to the increase of yield per unit of N rate, b refers to the N value in soil with the same unit as the N fertilizer rate, and M refers the maximum yield when the N rate is not limited. Significant interactions ($P \leq 0.05$) were observed between the two study locations, thus treatment means for each location are presented separately at each time.

Experiment 2: Integration of PL and inorganic N study

Data collected were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., 2013), where fertilization treatments were analyzed as fixed effects, while replication and locations were random effects as appropriate. Means were compared using the LSMEANS statement in PROC MIXED and the Tukey's honestly significant difference test 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 study locations, thus treatment means for each location are present separately at each time.

3.4 Results and Discussion

3.4.1 Weather Conditions

Weather conditions varied between the two locations of this study (Fig. 1). Average growing season air temperatures were 17.9 and 19.7 ºC for 2017 at EVS and PAU, respectively. Generally, monthly temperatures among growing seasons were normal and did not deviate much (more than 2-3 ºC) from the 30-yr average during the course of this study. Monthly precipitation data collected from EVS showed that totals were 1026 mm with 608 mm occurring during the

growing season (November to the beginning of June), while from the PAU showed that totals were 1232 mm with 779 mm occurring during the growing season. Precipitation data here are for 2016 to 2017 at both locations. Precipitation during the germination period was very low with 5.8 and 8.6 mm of rainfall occurring at the EVS and PAU, respectively, thus, irrigation was applied the first week after sowing at a rate of 12 mm per day to promote germination. Although both field locations had comparatively similar weather conditions, PAU had higher average temperature throughout the year and greater precipitation during the months of January and June in 2017.

3.4.2 Canola Growth and Grain Yield Response to N Rate

Plant growth

Plant height tended to increase with increasing N rate (Table 3). At the rosette stage (T1, the end of Feb.), rates of 180 kg N ha^{-1} and greater produced significantly taller plants than the unfertilized control at EVS ($P < 0.0001$), while rates of 135 kg N ha^{-1} and greater contributed to a significant increase in plant height compared to the unfertilized control at PAU ($P < 0.0001$). Once the plants reached the flowering stage (T2, the end of Mar.), plots receiving urea application exhibited significantly taller plants than the unfertilized control. Nevertheless, plots with 180 kg N ha^{-1} had the tallest plants with plant height increasing 43% compared to the control at EVS ($P < 0.0001$). While at PAU, applying 135 kg N ha^{-1} or more N fertilizer resulted in taller plants than the control, with plant height increasing with increasing N rate ($P < 0.0001$). At the seed-fill stage (T3, the end of Apr.), applying 270 kg N ha^{-1} resulted in the tallest plants, with height increasing 11 and 29% compared to the unfertilized control at EVS ($P < 0.0001$) and PAU ($P < 0.0001$), respectively. Ma et al. (2015) reported that plant height, which is genetically and environmentally determined, is an indicator of the vegetative growth and crop yield

potential. Therefore, based on these results applying at least 135 or 180 kg N ha^{-1} enhances plant height and is needed to optimize yields.

The leaf greenness (chlorophyll concentration) decreased with increasing N rate at the rosette stage at EVS ($P = 0.0006$) and PAU ($P = 0.0025$), and the no N treatment had the highest SPAD readings at both locations (Table 3). While at the flowering stage, leaf greenness was minimally affected by N rates at EVS ($P = 0.7417$) and PAU ($P = 0.3681$) and seemed to have had no clear relationship to N rate (Table 3). Although, N plays an important role in the production of chlorophyll, the dilution effect caused by plant height, leaf numbers, and leaf area may also influence the chlorophyll concentration.

Although plant fresh ($P = 0.1803$) and dry ($P = 0.1978$) biomass production was not greatly influenced by N fertilization at EVS, the plots receiving a higher rate of N fertilizer tended to have greater fresh and dry aboveground biomass (Table 4). Unlike EVS, at the PAU location applying 135 kg N ha^{-1} and more had significantly greater fresh biomass than the unfertilized control ($P = 0.0007$), with an increase of 90, 66, 86, and 109% for the rates of 135, 180, 202, and 270 kg N ha^{-1}, respectively (Table 4). Plants receiving 135, 202, and 270 kg N ha^{-1} had 67, 81, and 85%, respectively greater dry biomass than that with no N application treatment at PAU ($P = 0.0057$). These results demonstrate that the application of at least 135 kg N ha^{-1} urea could result in greater aboveground biomass for canola as compared to the unfertilized control.

The concentration of N in plant samples was affected by the N rate applied at both locations (Table 4). Applying 135, 202, and 270 kg N ha^{-1} had significantly greater N concentrations at EVS ($P = 0.0054$), while at PAU, only the application of 180 and 270 kg N ha^{-1} had greater N concentration than the unfertilized control ($P = 0.0004$). Similarly, plants receiving more N fertilizer had greater plant N content at both locations (Table 4). A significantly greater plant N

content was observed in the plots with 202 and 270 kg N ha^{-1} at EVS ($P = 0.0247$), with an increase of 75 and 73%, respectively, compared to the control. At PAU, application of 180 kg N ha^{-1} and more urea had greater plant N content than the control ($P = 0.0005$) by 107% on average.

Grain yield and N uptake

The application of N fertilizer significantly increased the grain yield of canola at EVS ($P = 0.0003$) and PAU ($P < 0.0001$) (Fig. 2). A positive tendency was observed between grain yield and N rates. Our results are consistent with previous research showing that canola grain yield increased with increasing N fertilizer rates (Ozer 2003; Lafond 2004; Bishnoi et al. 2007; Karamanos et al. 2012; Zheljazkov et al. 2013; Al-Solaimani et al. 2015).

A significant increase of grain yield was observed in the plots that received 135 kg N ha^{-1} and more urea when compared to the control (no N treatment) at EVS location, while only the plots with more than 180 kg N ha^{-1} urea had greater grain yield than the control at PAU. However, no significant difference was observed for grain yield between the plots with 180 kg N ha^{-1} and more urea. Therefore, linear-plateau and exponential regression models were constructed to evaluate the relationship between N rate (X) and grain yield of canola (Y). The results indicate that canola grain yield and N rate were highly correlated based on either linear-plateau regression ($R^2_{adj} = 0.6713$, $P < 0.001$ for EVS and $R^2_{adj} = 0.7507$, $P < 0.001$ for PAU) or exponential regression ($R^2_{adj} = 0.7084$, $P < 0.001$ for EVS and $R^2_{adj} = 0.7367$, $P < 0.001$ for PAU). The linear-plateau regression equations are: $Y = 1372.46 + 4.85X$ ($X \leq 197$), $Y = 2327.70$ ($X > 197$) for EVS and $Y = 168.63 + 8.71X$ ($X \leq 232$), $Y = 2189.67$ ($X > 232$) for PAU. This model suggests that the optimal N rates needed are 197 and 232 kg ha^{-1} to obtain the maximum grain yields of 2328 and 2190 kg ha^{-1} at EVS and PAU, respectively. However, the optimal N

rate is greater than the recommended N rate (180 kg N ha^{-1}) based on soil testing and economic performance for both locations. Khakbazan et al. (2014) evaluated the economic effects of canola and N rate and reported the unit price of crop product as \$ 0.490 kg^{-1}, but the price of urea was \$ 0.63 kg^{-1}. Thus, differences in grain yield of canola between the recommended N rate (180 kg N ha^{-1}) and modeled optimal N rate (197 and 232 kg N ha^{-1}) indicated that the lower nitrogen rate is economical and more profitable than the higher application rate. Similar results were also reported by Bishnoi et al. (2007) for canola production in the southeastern US. The exponential regression equations are: $Y = 3009 \times [1 - e^{-0.004 \times (149 + X)}]$ for EVS and $Y = 33650 \times [1 - e^{-0.00024 \times (28.76 + X)}]$ for PAU. This model showed the maximum yield when the N rate is not limited (EVS is 3009 kg ha^{-1} and PAU is 33650 kg ha^{-1}) and the increase of yield per unit of N rate (EVS is 0.004 kg per unit N and PAU is 0.0002 kg per unit N). Thus, compared to EVS, PAU potentially produced larger yield but also requires more N input. Jackson (2000) reported that with spring canola, optimal seed yield occurred with 180 to 220 kg N ha^{-1}, and the linear relationship between total plant yield and N rates indicates that prolific growth of spring canola can occur when N supply is unlimited.

The application of N fertilizer significantly increased grain N uptake of canola at EVS ($P < 0.0001$) and PAU ($P < 0.0001$) (Fig. 3). A significant increase of grain N uptake was observed in the plots that received 135 kg N ha^{-1} and more urea, compared to the no N control treatment at the EVS location, while only the plots with more than 180 kg N ha^{-1} urea had greater grain N uptake than the control at PAU. Similar findings from increases in N rates have been reported by Jackson (2000). Linear-plateau and exponential regression models were constructed to evaluate the relationship between N rate (X) and N uptake of canola (Y). The results indicate that canola grain yield and N rate were highly correlated based on either linear-plateau regression (R^2_{adj} =

0.7542, $P < 0.001$ for EVS and $R^2_{adj} = 0.7607$, $P < 0.001$ for PAU) or exponential regression ($R^2_{adj} = 0.7561$, $P < 0.001$ for EVS and $R^2_{adj} = 0.7370$, $P < 0.001$ for PAU). The linear-plateau regression equations are: $Y = 45.85 + 0.19X$ ($X \leq 263$), $Y = 95.43$ ($X > 263$) for EVS and $Y = 8.10 + 0.40X$ ($X \leq 222$), $Y = 97.42$ ($X > 222$) for PAU. Maximum N uptake were similar for these two locations, but EVS needed an additional 40 kg N ha^{-1} compared to PAU. The exponential regression equations are: $Y = 206 \times [1 - e^{-0.0014 \times (179.7 + X)}]$ for EVS and $Y = 975 \times [1 - e^{-0.00038 \times (31.72 + X)}]$ for PAU. Similar to the grain yield observed from EVS, plants at PAU potentially took up more N, but also needed greater N input.

3.4.3 Effect of PL on Canola Growth and Grain Yield

Plant growth

Plant height can be used as an indicator of the vegetative growth and crop yield potential of a crop and is also genetically and environmentally determined (Ma et al. 2015). In this study, canola plant height responded positively to N fertilization (Table 5) when compared to the control (no N fertilization, P_0U_0). The PL only treatment produced significantly taller plants than those of the PL plus urea application treatments ($P_{68}U_{112}$ at EVS and $P_{68}U_{112}$ and $P_{112}U_{68}$ at PAU) and unfertilized control at the rosette stage (T1, the end of Feb.). Once canola plants reached flowering (T2, the end of Mar.) and the seed-fill stage (T3, the end of Apr.), the increase in plant height observed during the rosette stage was no longer evident as compared to the other N fertilization treatments. Significantly lower plant height was observed with the PL only application when compared to urea only or urea plus PL combination applications at the PAU during the seed-fill stage (T3). In contrast, combining PL plus urea application, especially the $P_{68}U_{112}$ did not produce taller plants at the T1 and T2 vegetative growth stages, but had increased plant height 7.5 and 24.6% by the time it reached the seed-fill stage (T3) when compared to the

unfertilized control at EVS and PAU, respectively. This may be due to the slow mineralization of litter-N not being able to satisfy the high requirement of N needed during the reproductive stages, while the urea N was more readily available during these growing periods to improve plant growth. Our results were consistent to a previous study, which showed that wheat fertilized with 90 kg N ha^{-1} PL plus 45 kg N ha^{-1} inorganic N fertilizer did not result in taller plants at the tillering stage when compared to the recommended rate of inorganic N fertilizer. However, the 90 kg N ha^{-1} PL plus 45 kg N ha^{-1} inorganic N fertilizer treatment significantly increased plant height at the late stem extension and late heading stages, while the single PL application only had taller plants at the tiller stage, when compared to the recommended inorganic N fertilization rate (Lin et al. 2017). Leaf greenness (chlorophyll concentration) was minimally affected by PL or urea applications relative to the unfertilized control at the various growth stages (Table 2). Addition of both PL and urea (both $P_{68}U_{112}$ and $P_{112}U_{68}$) seemed to have a relatively greater SPAD reading than the other treatments, albeit not significant, especially at the PAU location. Our results were inconsistent with Tewolde et al. (2010), which reported that PL application resulted in much lower leaf chlorophyll index than inorganic N application during the cotton growth stages. This may due to N from the inorganic fertilizer being readily available at the time of application, whereas only a small percentage of litter-N is immediately available for plant uptake. Although N plays an important role in producing chlorophyll, the growth parameters such as plant height, leaf numbers, and leaf area also influence the chlorophyll concentration by the dilution effect. For example, if two plants have the same chlorophyll content, the plant with greater plant height, more leaf numbers, or greater leaf area would have a lower chlorophyll concentration.

Nitrogen fertilization treatments at the EVS location had minimal influence on plant biomass (Table 6), however the plots with no N fertilizer had the lowest fresh and dry aboveground biomass. Adding both 68 kg N ha^{-1} PL and 112 kg N ha^{-1} urea resulted in a similar fresh biomass to the inorganic N treatment (P_0U_{180}), and had slightly greater dry biomass than the PL only treatment ($P_{180}U_0$) at the EVS location. Unlike, at the PAU location, both PL and urea combination application treatments ($P_{68}U_{112}$ and $P_{112}U_{68}$) had similar fresh biomass to that of the inorganic N application, and significantly increased fresh biomass when compared to the unfertilized control (Table 6), with an increase of 56.6% for the $P_{112}U_{68}$ treatment and 77.0% for the $P_{68}U_{112}$ treatment. Moreover, plants receiving both 68 kg N ha^{-1} PL and 112 kg N ha^{-1} urea application had significantly greater dry biomass, with a 59.3% increase, than that with no N application at the PAU location. The increases in growth characteristics (e.g. plant height, leaf greenness) and plant biomass with the PL plus urea combination might be due to the role of mineral N (more available) from inorganic fertilizer and macro- and micronutrients from PL stimulating the vegetative growth. These results demonstrate that combination of both 68 kg N ha^{-1} PL and 112 kg N ha^{-1} urea could result in similar or greater effects on canola growth as compared to inorganic N fertilizer.

Grain yield and N uptake

Application of N fertilizer significantly increased the grain yield of canola for all N sources at the PAU location (Table 6). Although there were no significant difference between the PL or urea treatment, the highest grain yield was observed with the urea only application followed by the $P_{68}U_{112}$ and $P_{112}U_{68}$ treatments, and the lowest grain yield was observed with the PL only application at the PAU location. A significant increase in grain yield was observed from plots receiving 68 kg N ha^{-1} PL plus 112 kg N ha^{-1} urea and with urea applied at the recommended N

rate when compared to the control N treatment at the EVS location (Table 6). In addition, the $P_{68}U_{112}$ treatment had the highest grain yield with an increase of 73.4% as compared to the P_0U_0 treatment. Our results are consistent to previous studies which have reported that no significant differences were observed between manure and inorganic N applications on grain yield of canola (Stevenson et al. 1998; Gao et al. 2010). Moreover, Stevenson et al. (1998) reported that canola produced when only a high rate of manure (fresh and stockpiled cattle manure) was applied had similar grain yield to those of inorganic fertilizer application under no-till and conventional tillage systems. Combined application of farmyard manure, compost and inorganic fertilizer increased canola grain yield compared to a single fertilizer application (farmyard manure, compost, and inorganic fertilizer) by an average of 59, 49, and 37%, respectively (Mohammadi and Rokhzadi 2012). Using inorganic N fertilizer could provide available N immediately to the crop, while applying PL could provide other macro- and micronutrients for plant growth and a slow release of N over time. Moreover, poultry manure application can improve soil physical and chemical properties, making suitable conditions for root development (Olatunji et al. 2012; Busari and Salako 2015).

Nitrogen content of the canola was estimated for the aboveground plant samples and seeds (Figs. 4 and 5). Nitrogen fertilization had no significant effect on plant N content at the EVS location (Fig. 4), although the $P_{112}U_{68}$ treatment numerically had the greatest N content followed to the urea only application and combination of 68 kg N ha^{-1} PL plus 112 kg N ha^{-1} urea treatment. At the PAU location, only plots with inorganic N application significantly increased plant N content compared to the control N treatment (Fig. 4). The $P_{68}U_{112}$ treatment had a similar N content to the inorganic N only treatment, although there were no significant differences between the $P_{68}U_{112}$ and no N treatments. The application of N fertilizer also significantly

increased N uptake in canola seeds at the PAU location (Fig. 5). The greatest N uptake was observed in plots receiving inorganic N only, followed by the combination of PL plus urea applications ($P_{68}U_{112}$ and $P_{112}U_{68}$), plots with PL application only had the lowest N uptake when compared to the other N fertilization treatments at the PAU location. Application of 68 kg N ha^{-1} PL plus 112 kg N ha^{-1} urea significantly increased the N uptake, with an increase of 82.0% when compared to the unfertilized control at the EVS location (Fig. 5). This is most likely due to the application of PL along with inorganic N fertilizer providing higher available N than a single application of PL or inorganic N. For example, Garrity and Flinn (1987) reported that integration of PL and inorganic fertilizer improved nutrient availability and soil conditions for plant growth by reducing the loss of nutrients, leading to a greater yield. Moreover, the use of poultry manure can enhance soil microbial enzyme activity and soil N availability for plants (Acosta-Martínez and Harmel 2006; Mankolo et al. 2012). In addition, the N content of a plant is relative to its biomass and grain yield. In this study, greater biomass and yield were observed in the P_0U_{180} and $P_{68}U_{112}$ treatments, which are likely to have a higher N content than the other treatments. Although the biomass of $P_{112}U_{68}$ treatment at the EVS location was relatively lower than the P_0U_{180} and $P_{68}U_{112}$ treatments, the greater N content observed in $P_{112}U_{68}$ treatment was mainly due to the high concentration of N in plant tissues (stem and leaves). Consistent with previous studies (Naher et al. 2016; Moe et al. 2017), these results indicate that a combination of PL and inorganic fertilizer can increase total N uptake and improve N use efficiency. These results also indicate that applying PL to winter canola in the short term may not satisfy plant N requirements due to the slow mineralization of litter-N, while a combination of PL and inorganic N could supply sufficient N as well as other nutrients needed for optimizing canola production.

3.5 Conclusion

Canola growth, yield, and N uptake are highly dependent on N fertility. Plant height, aboveground biomass, grain yield, and N uptake increased with the increasing N rate at both locations. Peak grain yields of 2190 to 2328 kg ha^{-1} occurred with N applications of 197 to 232 kg N ha^{-1}, suggesting that canola requires 0.08 to 0.11 kg N kg^{-1} of yield in the southeastern region.

Compared to inorganic fertilizer, applying only PL did not provide comparable plant growth or grain yield to winter canola production in this study. Applying 68 kg N ha^{-1} PL plus 112 kg N ha^{-1} urea resulted in the improvement of plant growth and increased grain yield and N uptake from both the loamy sand (EVS) and sandy loam soils (PAU). Therefore, these results suggest that a combination of PL and inorganic N fertilizer could reduce the usage of chemical fertilizer without decreasing the yields of winter canola, thereby providing sustainable yield production for winter canola in the southeastern US. However, the effect of canola on the following crop's production is not clear. Future work will be conducted to evaluate canola's performance as a winter crop with PL applications under double-cropping systems.

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Table 1. Initial soil properties for EVS and PAU and poultry litter properties on a dry weight basis.

Property†	EVS soil	PAU soil	Poultry litter
pH (1:1 soil:water)	6.7	5.5	-
Moisture content (g kg^{-1})	-	-	221
Total C (g kg^{-1})	3.8	8.9	292
Total N (g kg^{-1})	0.50	1.00	24.4
C:N ratio	7.6	8.9	12.0
P (g kg^{-1})	0.02	0.02	15.0
K (g kg^{-1})	0.06	0.10	25.2
Ca (g kg^{-1})	0.35	0.35	31.3
Mg (g kg^{-1})	0.08	0.06	6.4
Na (g kg^{-1})	0.03	0.03	10.4
Cu (mg kg^{-1})	1.36	0.99	212
Fe (mg kg^{-1})	7.9	11.7	1381
Mn (mg kg^{-1})	7.8	59.8	411
Zn (mg kg^{-1})	2.9	1.9	443

†P, K, Ca, Mg, Na, Cu, Fe, Mn, and Zn values represent Mehlich-extractable nutrient concentrations for soil and total nutrient concentrations for poultry litter.

Table 2. Urea application rate and timing for the N rate study at EVS and PAU in 2016 to 2017.

N rate (kg ha^{-1})	Application time		
	At sowing	01 March	01 April
0	-	-	-
68	22.67	22.67	22.67
135	33.75	33.75	67.5
180	45	45	90
202	50.5	50.5	101
270	67.5	67.5	135

Table 3. Response of plant height (cm) and SPAD readings measured at the various growth stages as influenced by N rate at EVS and PAU.

Site	N rate (kg ha^{-1})	Plant height (cm)			SPAD readings	
		T1†	T2	T3	T1	T2
EVS	0	14.23 ± 0.79 C‡	58.18 ± 2.56 C	125.2 ± 2.07 B	58.38 ± 0.92 A	52.83 ± 1.25
	68	18.05 ± 0.78 AB	67.00 ± 1.90 B	126.2 ± 2.09 B	54.01 ± 0.80 BC	52.92 ± 1.05
	135	15.88 ± 0.81 BC	66.93 ± 1.84 B	131.8 ± 1.71 AB	57.14 ± 1.07 AB	52.11 ± 0.95
	180	20.99 ± 0.96 A	79.00 ± 1.93 A	134.9 ± 1.87 A	54.57 ± 1.02 ABC	51.46 ± 1.60
	202	19.30 ± 0.96 AB	73.03 ± 1.89 AB	136.2 ± 2.04 A	56.25 ± 0.85 ABC	53.78 ± 1.02
	270	19.48 ± 0.91 A	72.45 ± 2.01 AB	138.4 ± 2.13 A	53.04 ± 1.03 C	53.44 ± 0.93
PAU	0	13.13 ± 0.94 d	10.80 ± 0.74 c	94.88 ± 3.30 c	51.96 ± 1.00 a	58.16 ± 1.13
	68	16.10 ± 1.20 cd	13.48 ± 0.54 c	112.6 ± 2.31 b	51.42 ± 0.63 ab	57.39 ± 1.27
	135	17.66 ± 1.03 bc	16.78 ± 0.77 b	118.2 ± 1.83 ab	49.06 ± 1.41 abc	55.69 ± 0.88
	180	19.41 ± 1.02 bc	19.35 ± 0.75 b	118.6 ± 1.86 ab	48.89 ± 0.94 abc	55.92 ± 0.83
	202	21.91 ± 1.36 ab	18.88 ± 0.86 b	117.0 ± 2.22 ab	47.80 ± 0.82 bc	55.57 ± 0.88
	270	26.38 ± 1.29 a	23.40 ± 0.85 a	122.2 ± 2.43 a	47.20 ± 0.90 c	56.41 ± 0.89
		P > F (0.05)				
	EVS	<0.0001	<0.0001	<0.0001	0.0006	0.7417
	PAU	<0.0001	<0.0001	<0.0001	0.0025	0.3681

†T1, 28 Feb.; T2, 31 Mar.; T3, 30 Apr.; at T3, not enough leaves were left on the plants, thus no SPAD readings are presented at this stage.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$. Capital letters show multiple comparisons of treatments at EVS, and small letters show multiple comparisons of treatments at PAU.

Table 4. Effect of urea application on biomass (kg) and plant N concentrations (g kg^{-1}) at EVS and PAU.

Site	N rate (kg ha^{-1})	Fresh biomass (kg)	Dry biomass (kg)	N conc. (g kg^{-1})	N content (g)
EVS	0	1.76 ± 0.13	0.49 ± 0.03	12.4 ± 0.63 C	6.11 ± 0.63 B
	68	1.98 ± 0.20	0.56 ± 0.07	13.7 ± 0.98 BC	7.76 ± 1.26 AB
	135	2.23 ± 0.22	0.59 ± 0.08	14.9 ± 0.59 AB	8.71 ± 0.93 AB
	180	2.38 ± 0.28	0.63 ± 0.06	14.7 ± 0.87 ABC	9.20 ± 0.96 AB
	202	2.31 ± 0.32	0.66 ± 0.11	15.9 ± 1.03 AB	10.7 ± 2.21 A
	270	2.26 ± 0.17	0.63 ± 0.06	17.2 ± 1.38 A	10.6 ± 0.42 A
PAU	0	1.13 ± 0.12 c†	0.27 ± 0.02 b	15.8 ± 0.23 c	4.52 ± 0.43 c
	68	1.56 ± 0.18 bc	0.37 ± 0.04 ab	15.9 ± 0.18 bc	5.65 ± 0.89 bc
	135	2.15 ± 0.17 ab	0.45 ± 0.04 a	16.4 ± 0.26 bc	6.91 ± 0.47 bc
	180	1.88 ± 0.12 ab	0.42 ± 0.02 ab	19.4 ± 1.53 ab	8.16 ± 0.86 ab
	202	2.10 ± 0.23 ab	0.49 ± 0.07 a	18.2 ± 0.94 abc	8.95 ± 1.16 ab
	270	2.36 ± 0.14 a	0.50 ± 0.03 a	21.7 ± 0.89 a	10.9 ± 0.98 a
		P > F (0.05)			
	EVS	0.1803	0.1978	0.0054	0.0247
	PAU	0.0007	0.0057	0.0004	0.0005

†Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$. Capital letters show multiple comparisons of treatments at EVS, and small letters show multiple comparisons of treatments at PAU.

Table 5. Response of plant height (cm) and SPAD readings measured at the various growth stages as influenced by poultry litter and urea applications at EVS and PAU.

Site	Treatment†	Plant height (cm)			SPAD readings	
		T1	T2	T3	T1	T2
EVS	P_0U_{180}	20.99 ± 0.96 A‡	79.00 ± 1.93 A	134.9 ± 1.87 A	54.57 ± 1.02 AB	51.46 ± 1.60
	$P_{180}U_0$	20.51 ± 1.03 A	64.18 ± 2.00 BC	128.1 ± 2.24 AB	53.76 ± 1.03 B	53.58 ± 0.92
	$P_{112}U_{68}$	18.18 ± 0.87 AB	66.95 ± 2.18 B	128.1 ± 1.64 AB	53.95 ± 1.13 B	53.36 ± 1.02
	$P_{68}U_{112}$	17.08 ± 0.91 BC	69.95 ± 2.31 B	134.6 ± 1.70 A	56.78 ± 0.76 AB	53.63 ± 1.04
	P_0U_0	14.23 ± 0.79 C	58.18 ± 2.56 C	125.2 ± 2.07 B	58.38 ± 0.92 A	52.83 ± 1.25
PAU	P_0U_{180}	19.41 ± 1.02 ab	19.35 ± 0.75 a	118.6 ± 1.86 a	48.89 ± 0.94	55.92 ± 0.83
	$P_{180}U_0$	20.7 ± 1.06 a	13.7 ± 0.59 b	95.60 ± 3.07 b	47.77 ± 0.83	55.75 ± 1.04
	$P_{112}U_{68}$	16.8 ± 1.13 bc	11.13 ± 0.49 bc	111.3 ± 1.82 a	50.56 ± 1.00	58.49 ± 0.86
	$P_{68}U_{112}$	15.94 ± 1.00 bc	18.05 ± 0.74 a	118.2 ± 2.14 a	55.38 ± 8.25	57.59 ± 1.01
	P_0U_0	13.13 ± 0.94 c	10.80 ± 0.74 c	94.88 ± 3.30 b	51.96 ± 1.00	58.16 ± 1.13
		P > F (0.05)				
EVS		<0.0001	<0.0001	0.0006	0.0207	0.6768
PAU		<0.0001	<0.0001	<0.0001	0.6528	0.1478

†P_0U_0-unfertilized control; P_0U_{180}-urea at 180 kg N ha^{-1}; $P_{68}U_{112}$-PL at the rate of 68 kg total N ha^{-1} plus 112 kg ha^{-1} inorganic N $P_{112}U_{68}$-PL at the rate of 112 kg total N ha^{-1} plus 68 kg ha^{-1} inorganic N; and $P_{180}U_0$-PL at the rate of 180 kg total N ha^{-1}. T1, 28 Feb.; T2, 30 Mar.; T3, 30 Apr.; at T3, not enough leaves were left on the plants, thus no SPAD readings presented at this stage.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$. Capital letters show multiple comparisons of treatments at EVS, and small letters show multiple comparisons of treatments at PAU.

Table 6. Effect of poultry litter and urea applications on biomass (kg) and grain yield (kg ha^{-1}) of canola at EVS and PAU.

Site	Treatment†	Fresh biomass (kg)	Dry biomass (kg)	Grain yield (kg ha^{-1})
EVS	P_0U_{180}	2.38 ± 0.28	0.63 ± 0.06	2234 ± 208 A
	$P_{180}U_0$	1.93 ± 0.24	0.53 ± 0.08	1480 ± 269 B
	$P_{112}U_{68}$	2.19 ± 0.28	0.63 ± 0.09	1830 ± 87.9 AB
	$P_{68}U_{112}$	2.28 ± 0.42	0.65 ± 0.12	2287 ± 26.9 A
	P_0U_0	1.76 ± 0.13	0.49 ± 0.03	1319 ± 111 B
PAU	P_0U_{180}	1.88 ± 0.12 ab‡	0.42 ± 0.02 a	2002 ± 285 a
	$P_{180}U_0$	1.48 ± 0.19 bc	0.35 ± 0.05 ab	1327 ± 97.7 a
	$P_{112}U_{68}$	1.77 ± 0.15 ab	0.38 ± 0.04 ab	1491 ± 31.8 a
	$P_{68}U_{112}$	2.00 ± 0.18 a	0.43 ± 0.05 a	1637 ± 225 a
	P_0U_0	1.13 ± 0.12 c	0.27 ± 0.02 b	366.2 ± 89.0 b
		P > F (0.05)		
EVS		0.4244	0.49	0.0011
PAU		0.0007	0.0248	0.0005

†P_0U_0-unfertilized control; P_0U_{180}-urea at 180 kg N ha^{-1}; $P_{68}U_{112}$-PL at the rate of 68 kg total N ha^{-1} plus 112 kg ha^{-1} inorganic N $P_{112}U_{68}$-PL at the rate of 112 kg total N ha^{-1} plus 68 kg ha^{-1} inorganic N; and $P_{180}U_0$-PL at the rate of 180 kg total N ha^{-1}.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$. Capital letters show multiple comparisons of treatments at EVS, and small letters show multiple comparisons of treatments at PAU.

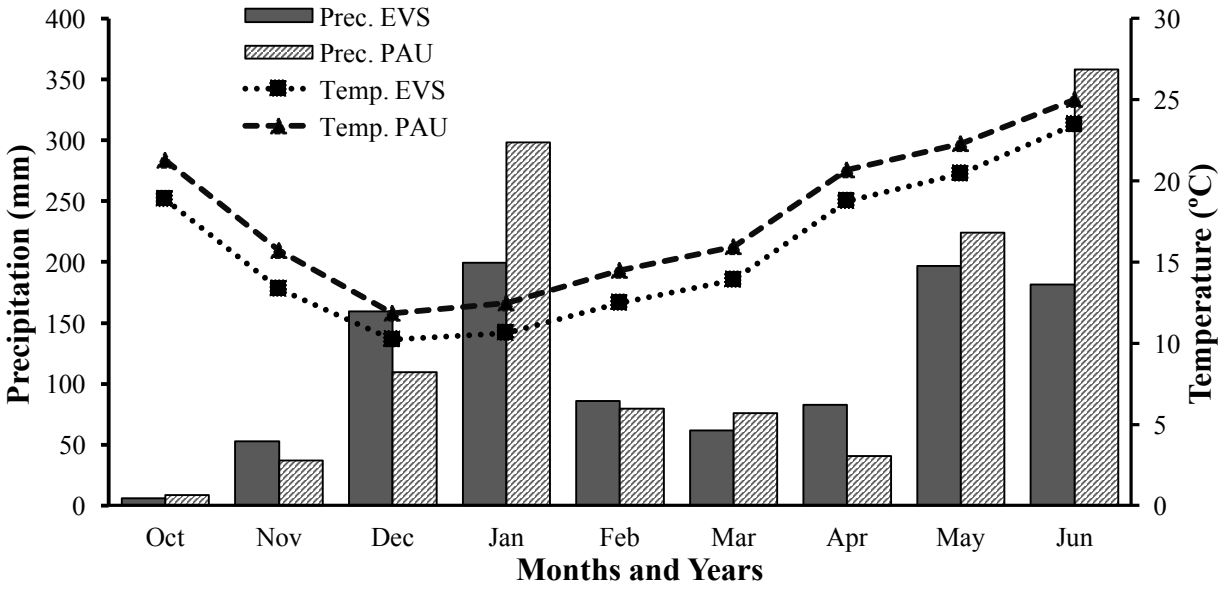

Fig. 1. Monthly average air temperature and precipitation totals at the Alabama Agricultural Experiment Station's E. V. Smith Research Center (EVS) and Prattville Agricultural Research Unit (PAU) for October 2016 to June 2017.

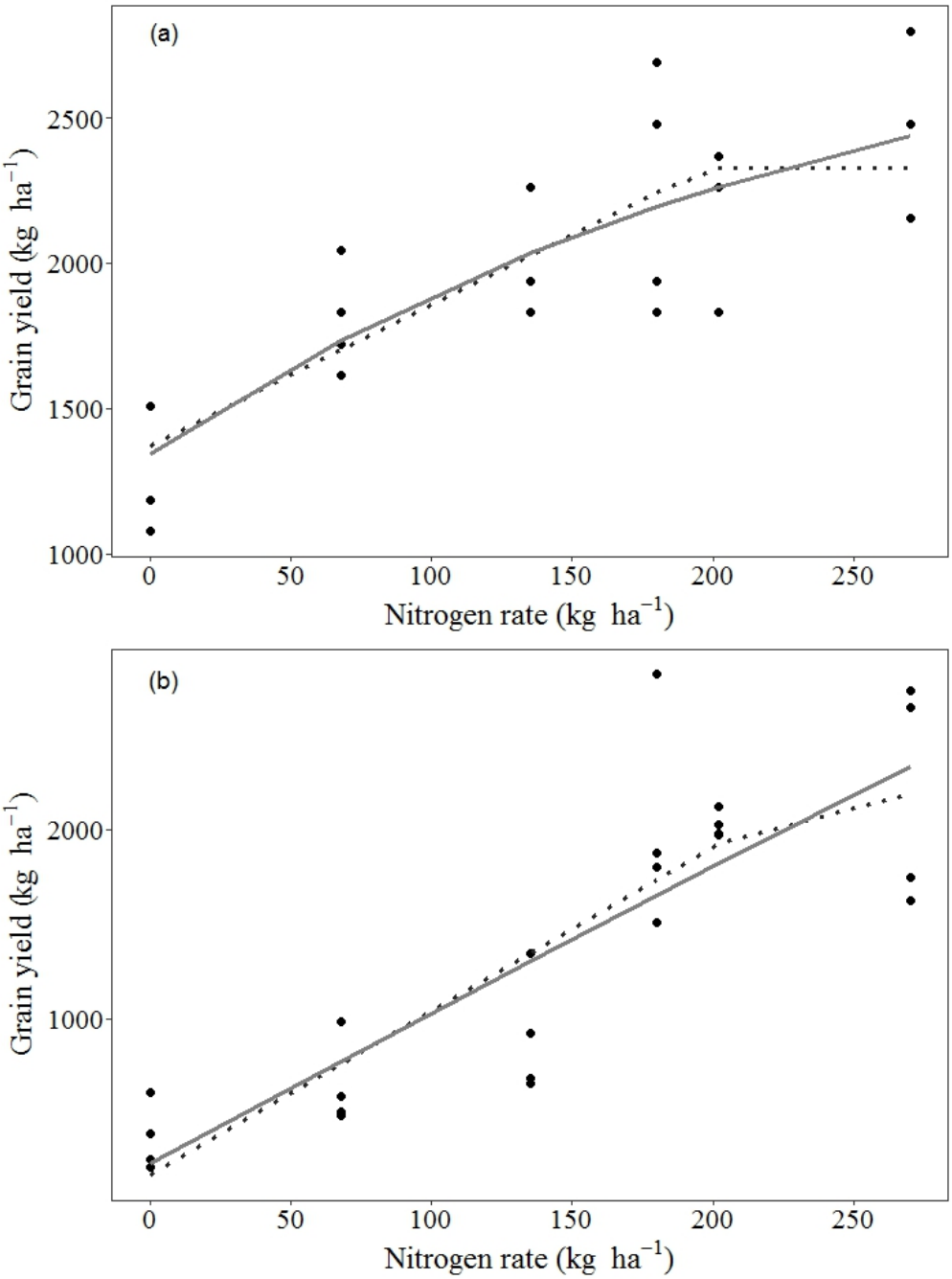

Fig. 2. The relationship between N rate and grain yield for canola at (a) EVS and (b) PAU locations. Dashed line is the linear-plateau regression and solid line is the exponential regression.

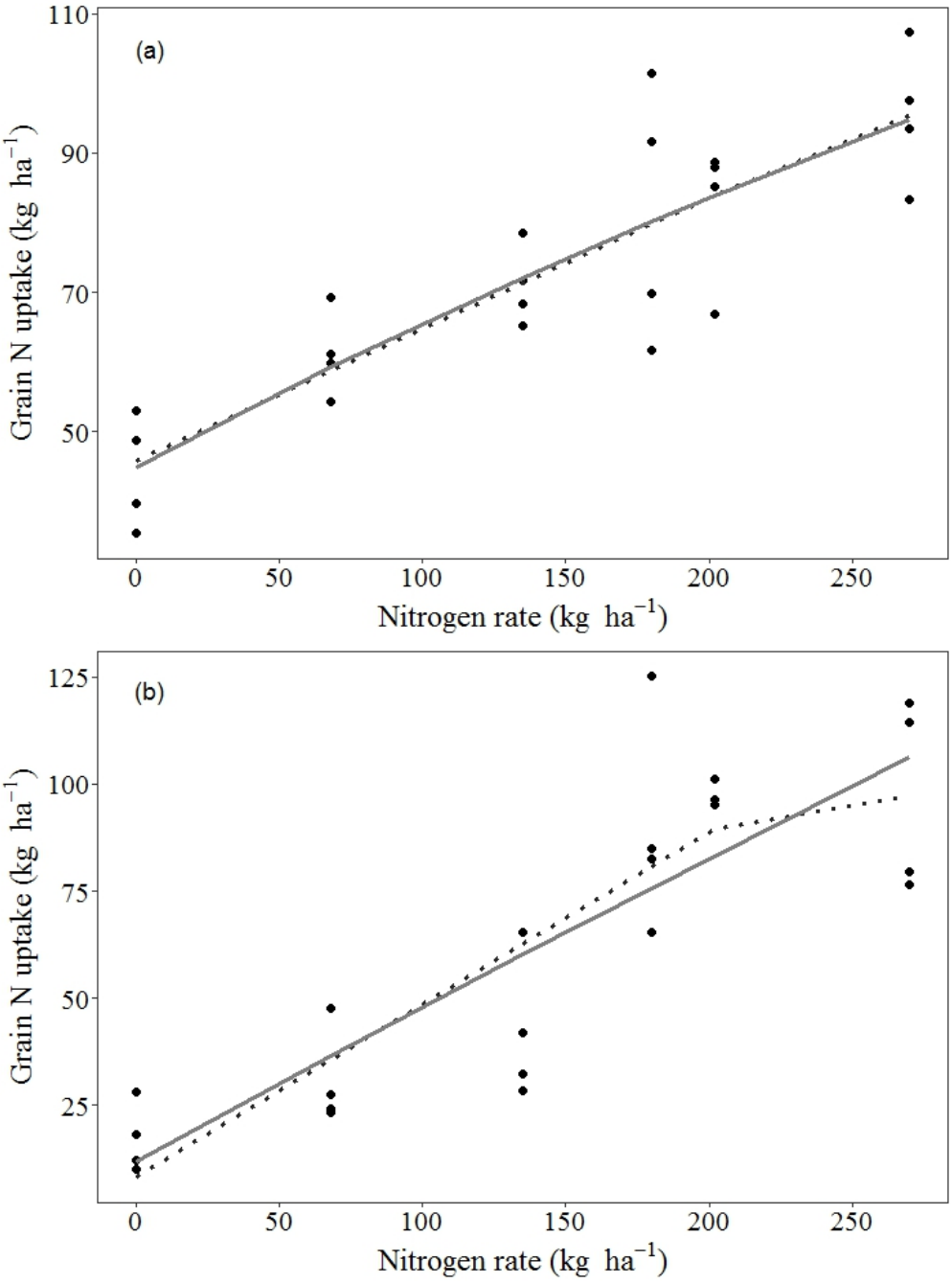

Fig. 3. The relationship between N rate and N uptake for the canola seed yield at (a) EVS and (b) PAU locations. Dashed line is the linear-plateau regression and solid line is the exponential regression.

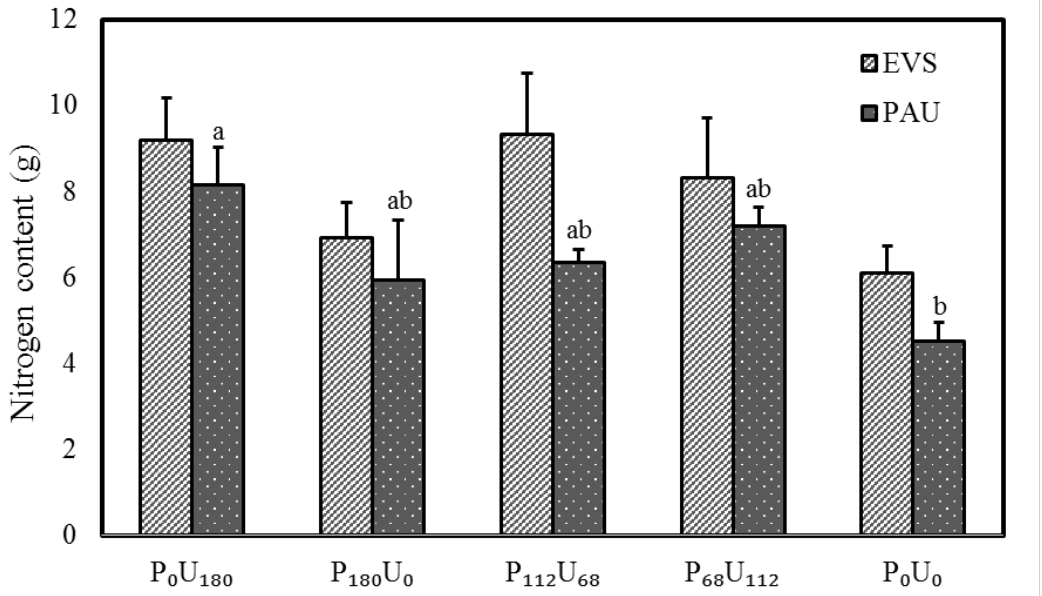

Fig. 4. Nitrogen content of canola plant samples at the Alabama Agricultural Experiment Station's E. V. Smith Research Center (EVS) and Prattville Agricultural Research Unit (PAU). Data represent means and standard errors of replicates. Within each location, bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$.

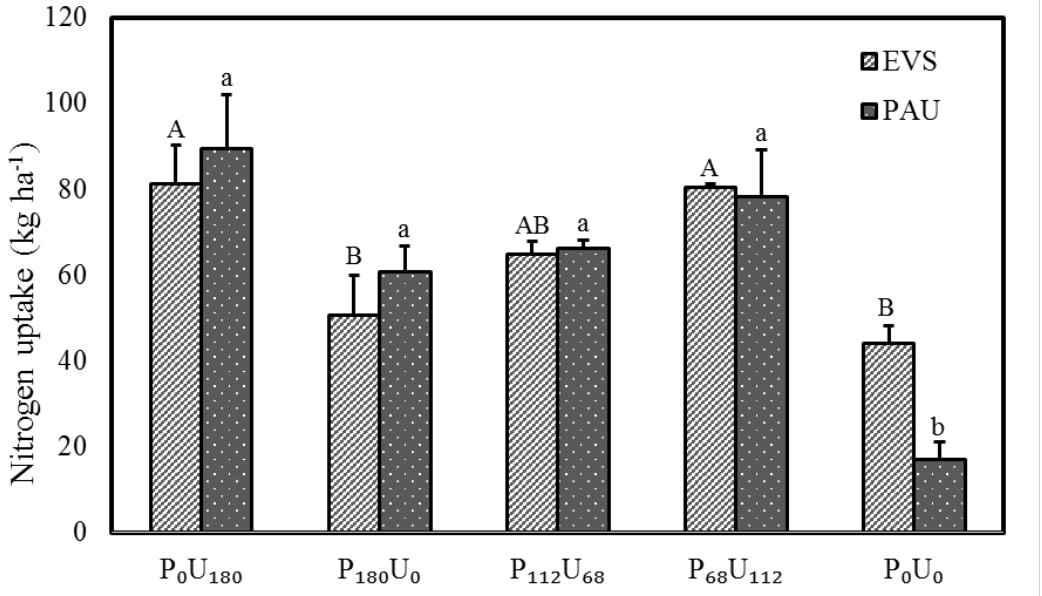

Fig. 5. Nitrogen uptake for canola seed yield at the Alabama Agricultural Experiment Station's E. V. Smith Research Center (EVS) and Prattville Agricultural Research Unit (PAU). Data represent means and standard errors of replicates. Within each location, bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$, where capital letters show multiple comparisons of treatments at EVS, and small letters show multiple comparisons of treatments at PAU.

4. Effect of PGPR on Corn Growth under Different Fertility Sources

4.1 Abstract

Free-living bacteria in the plant rhizosphere can mediate soil processes such as nitrogen fixation, mineralization, solubilization, and nutrient mobilization. Thus, inoculating seeds with bacteria known to promote plant growth may be a promising technique for enhancing nutrient-use efficiency and improving crop production. Accordingly, a study was conducted to evaluate the effects of plant growth-promoting rhizobacteria (PGPR) on root establishment and biomass production of corn (*Zea mays* L.) during the early growth stages using three fertility sources under glasshouse conditions. Treatments included three fertility sources (poultry litter (PL), biosolids, and urea) at 168 kg total N ha^{-1} and five PGPR inoculants (4 PGPR strain mixtures and 1 control without PGPR). Applying PL significantly improved root morphological parameters and increased plant biomass at the V4, V6, and VT growth stages when compared to the other fertility sources. At the V4 stage, PGPR stimulated root growth and enhanced aboveground biomass with urea and PL, while no differences were observed with PGPR and biosolids. At the V6 stage, PL, biosolids, and urea with PGPR significantly increased some growth parameters (e.g., plant height, leaf area, and root morphology). However, at the VT stage, PGPR's influence on plant growth was minimal regardless of fertility source. Applying the fertility sources at 135 kg N ha^{-1} may have masked PGPR's influence on corn growth as the plants reached their later vegetative growth stages. Future research is needed to evaluate the influence of PGPR on plant growth when fertility requirements are not optimal.

4.2 Introduction

The extensive use of chemical fertilizers to satisfy the increasing demand for food is not only costly, but can lead to potentially serious environmental problems. Therefore, the worldwide use

of organic and bio-fertilizers for sustainable agriculture has increased in efforts to improve soil properties and increase nutrient use efficiency.

Plant growth-promoting rhizobacteria (PGPR) use as biofertilizers can increase soil productivity and plant growth due to their interactions within the soil-plant system. (Yazdani et al. 2009). These bacteria are capable of colonizing plant roots and promoting plant growth by producing and secreting various chemical regulators in the rhizosphere. These mechanisms include nitrogen fixation, solubilization of inorganic phosphate, mineralization of organic phosphate, production of plant growth regulators or phytohormones, synthesis of siderophores, and indirect catalytic actions (Zafar et al. 2012). Numerous studies have indicated that PGPR can stimulate seed germination (Gholami et al. 2009; Noumavo et al. 2013), strengthen plant stems (Çakmakçi et al. 2006; Jarak et al. 2012; Sengupta et al. 2015), increase the leaf area and the number of leaves (Çakmakçi et al. 2006; Nezarat and Gholami 2009; Sengupta et al. 2015), and stimulate root growth (Jacoud et al. 1999; El Zemrany et al. 2007). As a result, increased biomass or yield has been observed for cotton (*Gossypium hirsutum* L., Egamberdiyeva and Hoflich 2004; Xiang et al. 2017), soybean (*Glycine max* (L.), Zhang et al. 1997; Cassán et al. 2009), corn (Shaharoona et al. 2006; Biari et al. 2008, Couillerot et al. 2013), rice (*Oryza sativa*, Biswas et al. 2000; Cong et al. 2009), peanut (*Arachis hypogaea* L., Dey et al. 2004), ryegrass (*Lolium multiflorum* L., Yolcu et al. 2011), and wheat (*Triticum aestivum* L., Díaz-Zorita and Fernández-Canigia 2009; Billah and Bano 2015).

Among the PGPR microorganisms, the bacilli (species of *Bacillus* and related genera previously classified as *Bacillus*) are the most widely used bacterial inoculants, which have wide metabolic capabilities allowing them to play important roles in soil ecosystem functions and processes. Due to their heterotrophic nature, the bacilli are also important in soil carbon (C),

nitrogen (N), and sulfur (S) cycling, as well as the transformation of other soil nutrients (Mandic-Mulec and Prosser 2011). Previous studies and reviews have reported plant growth promotion, increased yield, phytohormone production, P solubilization, N uptake, and other ions following inoculation with species of *Bacillus.* (Singh and Kapoor 1999; Dobbelaere et al. 2003; Çakmakçi et al. 2006; Hayat et al. 2010; Wani and Khan 2010).

The addition of organic fertility sources such as manure and biosolids to soil not only enhances crop yields, but may also play an important role in minimizing fertilizer nutrient losses from runoff and leaching, while retaining soil moisture and nurturing soil microbes (Bot and Benites 2005). Using poultry litter (PL) as a low-cost nutrient source has increased in recent years among row crop producers in the southeastern U.S. because of its increased availability and its potential for improving crop production (Reddy et al. 2004; Hirzel et al. 2007; Mitchell and Tu 2005; Tewolde et al. 2009; Watts and Torbert 2011). Results from a long-term corn-soybean rotation experiment indicate that PL amendments to a conservation tillage system increased the CEC, organic matter, and macro- and micro-nutrients in soil (Watts et al. 2010), thereby, significantly improving corn and soybean yields (Watts and Torbert 2011). Mitchell and Tu (2005) showed that compared with ammonium nitrate (NH_4NO_3), long-term broiler litter applications increased cotton lint yield by 30 to 50% and corn grain yield by 25 to 65% when applied at the same total N rate. Jn-Baptiste et al. (2013) observed similar results for corn production, with yield averages with PL increasing up to 800 kg ha^{-1} greater than the application of NH_4NO_3 at the same available N rate (assuming a 50% N availability rate). Biosolids have also been applied to agricultural lands as a cost-effective alternative to commercial fertilizer or as a soil amendment since they are rich in organic matter and contain a substantial amount of macro- and micro-nutrients (Hargreaves et al. 2008; Lu et al. 2012; Shaheen and Tsadilas 2013;

Torrecillas et al. 2013). Codling (2014) found that wheat biomass yield increased with biosolid applications into Minnesota and Maryland soils when compared to the non-biosolid-applied control. A long-term tall fescue (*Festuca arundinacea* Schreb.) experiment in Washington showed that surface application of biosolids resulted in slightly higher forage yields compared to NH_4NO_3 during the 10 years of applications (Cogger et al. 2013). After these applications ceased, yields from the biosolid treatments remained significantly greater than from NH_4NO_3 treatment in the succeeding 9 years when the residual effects were evaluated (Cogger et al. 2013).

Several studies have reported that soil ecological processes and indicators, such as microbial activities, biomass and diversity, and enzyme activities, are influenced by organic amendments (Parham et al. 2003; Liu et al. 2010; Lu et al. 2012; Fereidooni et al. 2013). In addition, applications of effective cultures of microorganisms to soil can stimulate the decomposition of organic wastes and residues, thereby releasing inorganic nutrients that become available for uptake by the plants. Biochar application with *Bacillus* sp. increased N and P content of French beans (*Phaseolus vulgaris*), compared to the same rate of chemical fertilizer with inoculants (Saxena et al. 2013). *Pseudomonas fluorescens* supplemented with a half dose of N-organic fertilizer significantly increased the growth and yield of corn compared to the full dose of chemical N fertilizer (Naveed et al. 2008). In contrast, some studies demonstrated that a combination of PGPR with both chemical and organic fertilizers resulted in the greatest yields. For example, under salt-affected conditions, PGPR inoculation with a half dose of chemical N and slurry resulting from biogas production, enhanced growth, yield, and nutrient concentrations of corn, and also improved soil pH, electrical conductivity (EC), and available N, P, and K content (Ahmad et al. 2014).

Limited research has been published on the influence that organic fertility sources have on the capacity of PGPR to increase plant biomass production and crop yield; specifically, the interaction between PGPR, PL, and biosolids. Therefore, the objective of this research was to investigate the effectiveness of PGPR mixtures on root establishment and biomass accumulation of corn when used with organic or inorganic fertility sources.

4.3 Materials and Methods

A glasshouse container study was conducted concurrently at Auburn University's Horticulture-Paterson Greenhouse Complex (HP) and Plant Science Research Center (PSRC) from June to August of 2016. The soil used for this study was a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult) collected from the Alabama Agricultural Experiment Station's E. V. Smith Research Center-Field Crops Unit in Macon County, near Shorter, AL. Surface soil (0-15 cm depth) was collected in early-spring from an area that had been previously under row crop production. The PL used for this study was collected from a local broiler producer using standard production practices and consisted of manure and a bedding material mixture of wood shavings. The biosolid (BS) used for this study was collected from a nearby municipality. Soil, PL, and biosolid used in this study were submitted to the Auburn University Soil Testing Laboratory and analyzed for nutrient concentrations (Table 1) according to procedures described by Hue and Evans (1986). Briefly, soil pH was determined on a 1:1 soil:water suspension using a glass electrode meter. Total C and N were determined by dry combustion using a LECO Truspec (LECO Corp., St. Joseph, MI, USA). Concentrations of P, K, Ca, Mg, Na, Cu, Fe, Mn, and Zn were determined using the dry ash procedure for PL (Donohue 1983) and with a Mehlich-1 extracting solution for soil (Olsen and Sommers 1982); both were measured using an ICAP 9000 (Thermo Jarrell Ash, Franklin, MA, USA).

The experimental design was a randomized complete block design with five replications for each treatment. Treatments consisted of three fertilizer sources combined with five PGPR inoculants. The three fertility sources consisted of PL (2.9% N) and BS (1.7% N), and urea (46% N) as inorganic fertilizer (IF). Each fertility source was applied at a rate of 135 kg total N ha^{-1} and was mixed with the top 15 cm of soil in the pot. PGPR treatments consisted of four PGPR strain mixtures (Table 2) and one control without PGPR. The strains from the culture collection of Auburn University were used in the study. These strains have been shown to have positive effects on plant growth when evaluated in previous screenings. The bacterial mixtures were prepared by mixing each spore suspension of each strain in equal concentrations to result in the bacterial mix at a final concentration of 1 x 10^6 cfu ml^{-1}.

The experimental units consisted of plastic containers (8 L Gro Pro square pots, Sunlight Supply, Inc., Vancouver, WA, USA) that were 24 cm tall, measuring 23 x 23 cm at the top, and tapered to 18 x 18 cm at the base. The containers were filled with 12.5 kg of soil and watered to saturation based on five extra containers designated for this purpose. 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 to a depth of 5 cm into the moist soil, and 1 ml of suspension of the respective bacterial mixture (*Bacillus* spp. and *Pseudomonas moraviensis* sp.) was applied on top of each seed at planting. After germination, plants were thinned to one plant per container and watered every three days to saturation. Temperature within the glasshouses was maintained at 24 °C during the day and 17 °C at night. The PSRC had fans that operated continuously to circulate air throughout the glasshouse, while the HP glasshouse did not.

The corn plants were harvested at V4, V6, and VT growth stages. Plant height, stem diameter, leaf area, root morphological features, and dry shoot and root weights were measured at each harvest growth stage. Plant height was determined by measuring from the plant's base to the top of the newest fully developed leaf. Afterwards, plants were cut at the soil surface with handheld pruning shears. Stem diameter was determined using high-precision digital calipers. Leaf area was determined from the harvested plants using an area meter (LI-3100C Area Meter, LI-COR Biosciences, Lincoln, NE, USA). For each plant, all leaves were cut from the stem 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. To determine dry weight, the above- or below-ground plant biomass was then placed into paper bags and dried in a forced-air drying oven at 55 ºC until the weight became constant. 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 10000 professional scanner (Seiko Epson Corp., Shinjuku, Tokyo, Japan). Each individual root system was evenly spread apart in a water bath on a transparent tray (30 x 40 cm width) and imaged at a resolution of 157.5 dots per cm as described by Bauhus and Messier (1999) and Costa et al. (2000). The following root characteristics were determined: total root length (cm), root surface area (cm^2), root volume (cm^3), and average root diameter (mm). Plant total C and N analyses were performed on the dried shoot and root tissues. Ground tissue from each plant leaf, stem, or root harvested at the VT stage was analyzed for C and N concentrations using the combustion method (LECO FP-528 Nitrogen/Protein Analyzer, LECO Corp., St. Joseph, MI, USA).

An analysis of variance (ANOVA), using a general linear model (GLM), 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 with SAS 9.4 (SAS Institute Inc. 2013). Significant interactions ($P \leq 0.05$) were observed between study locations and fertilizer sources. Thus, treatment means for IF, BS, and PL were analyzed separately at each location.

4.4 Results and Discussion

4.4.1 Plant Growth Promotion

The organic fertility sources (PL and BS) enhanced corn growth (Table 3 and 4) and biomass accumulation (Figs. 1 and 2) during the vegetative growth stages. Poultry litter application increased plant height (up to 28.6%), stem diameter (up to 55.9%), and leaf area (up to 80.1%) compared to IF application during the vegetative growth stages at the HP location (Table 3). Similarly, at the PSRC location, PL application increased plant height (up to 44.9%), stem diameter (up to 90.2%), and leaf area (up to 180%) compared to IF during the vegetative growth stages (Table 4). Likewise, BS application increased plant height (up to 34.1%), stem diameter (up to 42.7%) and leaf area (up to 83.7%) compared to the IF treatments at the PSRC location. The greatest biomass was obtained with plants where PL was incorporated into soil. At the HP location, biomass of the roots, stems, and leaves increased by 121%, 358%, and 211%, respectively when compared to the IF treatments at the V4 stage. However, the positive effects were reduced at the V6 stage, and no differences were observed at the VT stage (Fig. 1). Unlike the HP location, at the PSRC location both BS and PL applications had positive effects on stem and leaf growth during the vegetative growing stages (Fig. 2). Poultry litter application increased the root, stem, and leaf biomass by 61.4%, 339%, and 231%, respectively compared to IF treatments. Likewise, an increase in stem biomass by 113% and leaf biomass by 109% were observed with BS application at the PSRC.

Corn seeds inoculated with the different PGPR mixtures showed similar or greater plant growth parameters (Tables 3 and 4) and plant biomass (Figs. 1 and 2) when compared to non-inoculated seeds under different fertility sources during the growing season. At the HP location, the application of PGPR mixtures had no significant effects on stem diameter or leaf area but affected plant height and biomass accumulation at the V4 stage (Table 3 and Fig. 1a). PGPR mixture 2 with IF (IF-P2) had the highest plant height and greatest leaf area when compared to the other treatments at all three samplings. At the V4 stage, significant increases in leaf biomass of 36.4% ($P = 0.0308$) and root biomass of 56.4% ($P = 0.0415$) were observed after inoculation of PGPR mixture 2 when combined with IF application. PGPR mixture 2 also significantly increased plant height 9.5% compared to non-PGPR application, when combined with PL (PL-P2) at the V6 stage. Although the influence of PGPR on above-ground plant growth parameters varied with BS application at the different growth stages, there was no significant response to the application of PGPR mixtures during the corn vegetative growth stages at either location (Tables 3 and 4). The PGPR mixture 4 showed a significant positive effect on root biomass at only the VT stage, with an increase of 39.6% ($P = 0.0313$) with BS application compared to non-PGPR (BS-C) treatment at the PSRC location. The PGPR mixtures 1 and 3 had significantly higher stem biomass than the uninoculated plants ($P = 0.0066$) with IF application at the V4 stage. The IF-P1 treatment also increased the root biomass up to 47.4% compared to the non-PGPR application ($P = 0.0298$) in the same fertilized soils. There was no significant response to the application of PGPR mixtures on plant height and stem diameter regardless of fertilizer source at the PSRC location (Table 4), but a significant positive effect of PGPR inoculation was observed for leaf area, increasing 51.4% for PGPR mixture 1 at the V4 stage and 65.9% for PGPR 4 at the VT stage with IF application.

4.4.2 Root Morphology

By comparison with IF, the application of PL resulted in higher measures of some root morphological parameters for the three samplings at both locations, while BS application showed slightly lower root morphologies at the HP location (Table 5), but slightly increased root growth at the PSRC location (Table 6). At the VT stage, BS application significantly increased the average root diameter, root surface area, and total root volume by 25.4%, 45.7%, and 81.4%, respectively compared to the IF treatments. Poultry litter application increased total root length, average root diameter, root surface area, and total root volume up to 26.8%, 31.3%, 63.7%, and 112%, respectively compared to IF application at the HP location, while the effects were lower at the PSRC location.

The influence of PGPR inoculation on root morphological parameters were minimal compared to the differences observed among the fertility sources (Tables 5 and 6). The inoculation of the PGPR mixtures had no significant effect on root growth with PL, while effects were observed with IF application at the V4 stage and BS application at the V6 stage at both locations. With IF application, the PGPR mixture 2 significantly increased root diameter up to 14.6% and root volume up to 33.3% at the HP location, while PGPR mixture 1 performed the highest root surface area and total root volume at the PSRC location. An increased total root length of 12.2% was observed after the inoculation of the PGPR mixture 2 with BS fertilization at the HP location.

4.4.3 Carbon and Nitrogen Content

The organic fertility sources significantly affected corn C and N concentrations in the plant tissues (Tables 7 and 8). Both PL and BS applications had enhanced plant C concentrations, but lowered N concentrations, as compared to the IF treatments. On average, C concentrations of the

corn roots and stems increased up to 2.1% and 3.3% with BS, respectively compared to the IF at the PSRC location. Poultry litter increased root and stem C concentrations up to 2.7% and 1.9%, respectively compared to IF application at the PSRC location. The N content in all plant tissues under IF was significantly greater compared to that of the BS and PL treatments ($P < 0.001$), especially PL which resulted in the lowest N concentrations in corn tissues at both locations.

Carbon and N concentrations as affected by microbial inoculations for each of the fertility sources are shown in Tables 7 and 8. No significant effects of the PGPR on either corn tissue C or N concentration were observed from different fertility sources. However, the highest root and stem C concentration was obtained with the PGPR mixture 2 treatment with IF application, while PGPR mixtures 3 and 4 had the highest root and stem C concentrations with the BS. These two mixtures also had the highest root and leaf C concentrations when combined with PL. The response of corn N concentrations to PGPR inoculation varied depending on the fertility sources and plant tissues evaluated. Among the PGPR mixtures, 1 and 3 had higher N concentrations in most of the plant tissues with different fertility sources. For example, the highest stem and leaf N concentration was observed from plants treated with PGPR mixture 3 and PL application at the PSRC location, and also with IF application at the HP location.

4.4.4 Effects of Fertilizer on Corn Growth

Organic fertility sources such as manure and compost contain a variety of nutrients bound in different mineral and organic forms and are widely used in agriculture as low-cost alternatives to inorganic chemical fertilizers. In our study, PL increased corn above- and below-ground biomass at the V4, V6, and VT growth stages from both locations, while biosolid only increased the biomass at the PSRC location, but slightly decreased biomass at the HP when compared to the IF (Figs. 1 and 2). Although the response of corn aboveground growth (plant height, stem diameter,

and leaf area) and root growth to fertilizers (IF, BS, and PL) depended on the growth stage evaluated and PGPR strain inoculated, overall, PL and biosolid application improved corn aboveground and root growth at both locations (Tables 3-6). Our findings were consistent with those of other studies which reported that application of organic fertility sources resulted in an equivalent or better effect on crop growth and yield production (Mitchell and Tu 2005; Tewolde et al. 2009; Watts and Torbert 2011; Shaheen and Tsadilas 2013; Codling 2014). In our experiment, all fertilizer sources were applied at the same N rate and for IF treatments P and K were added based on fertilizer recommendation for the soil type. Thus, the increased growth and biomass accumulation for the corn could be attributed to macro- (except N, P, and K) and micro-nutrients in both the PL and biosolid, which improved the early-stage corn growth.

Results regarding the plant C and N concentrations (Tables 7 and 8) reveal that PL had lower root, stem, and leaf C and N concentrations (except root C concentration) at the PSRC location, while the BS resulted in higher root and stem C concentrations, but lower root, stem, and leaf N concentrations when compared to those of the IF treatment. Lower N concentrations in the tissues could be a result of a dilution effect from greater root biomass and also could be attributed to most of the N in PL and biosolid being in organic form, which is unavailable for plant uptake at the time of application. Similar results have been reported in several previous studies (Warman and Cooper 2000; Mitchell and Tu 2005; Tewolde et al. 2007; Tewolde et al. 2010). For example, Tewolde et al. (2010) indicated that fertilizing with PL resulted in significantly less N concentration in leaf, stem, and reproductive parts of cotton at the flowering and boll maturation stages when PL rates were compared to NH_4NO_3 application.

4.4.5 Effects of PGPR on Corn Growth

Plant growth-promoting rhizobacteria have various mechanisms for promoting plant growth, such as N fixation, P solubilization, and phytohormone production, which may increase the availability of soil nutrients to plants and improve plant growth. In our experiment, responses of corn growth to PGPR strains varied with growth stage and fertilizer type (Tables 3-6 and Figs. 1-2). During all three vegetative growth stages evaluated at both locations, inoculation with the PGPR mixtures significantly increased at least one plant growth parameter within at least one fertilizer source type. However, no general trend was observed for the PGPR mixtures to consistently increase plant growth among treatments when evaluating across all growth parameters. The greatest effects were observed at the V4 and V6 stages, with most growth parameters being significantly increased at these stages compared to the VT stage for the different fertility sources. Çakmakçi et al. (2006) reported that sugar beet (*Beta vulgaris*) plants inoculated with PGPR strains had a greater plant weight and a higher total sugar content at the early growth stage (60 DAP) than did plants fertilized with N or P, while the effect of the PGPR on growth appeared to have slowed down during the later growth stages when compared to N fertilizer. Similarly, Dobbelaere et al. (2002) reported a significant response from the microbial strains *Azospirillum brasilense* and *A. irakense* on plant and root dry weight, shoot length, and leaf length during the early-stage (30 DAP) growth of spring wheat. Our results are consistent with these findings in which early corn growth (V4 and V6) was more affected by PGPR inoculation than during the later stages (Tables 3-6 and Figs. 1-2). This may be because the phytohormones and plant growth regulators secreted by PGPR stimulated the seedling and early-stage growth of corn more when the requirement for nutrients was low. In contrast, a greenhouse pot study conducted by Calvo et al. (2017) showed that microbial-based treatments had greater and more consistent effects on corn growth and root development at the V6 and VT stages rather

than the V2 and V4 stages under different N fertilizers. Our results differed from this finding, since the influence of PGPR strains on plant growth were minimal regardless of the fertilizer type at the VT stage. The differences between the present study and Calvo et al. (2017) may be due to the differences in PGPR strains, soil conditions, and fertility sources between these two experiments. In addition, applying the fertility sources at the full N dose recommended (135 kg N ha^{-1}) might have masked PGPR's influence on corn growth as the plants reached their later vegetative growth stages.

Iqbal et al. (2016) reported that phosphate solubilizing bacteria strains were most effective for enhancing growth and yield of mung bean (*Vigna radiata*) in the presence of poultry manure compared to the absence of poultry manure, and indicated that the phosphatase activities of these bacteria largely contributed to the increases. Poultry litter contains all essential plant nutrients, and mixing PL into soil increased soil nutrient levels within the 0- 15 cm depth, thereby improving plant growth. Although the PL treatments in this experiment had greater plant growth than the other fertility sources, no obvious advantage was observed for most growth parameters with inoculation of PGPR with PL. As is known, the efficacy of PGPR inoculations on plant growth enhancement and crop yield depends upon its ability to survive and multiply in soils; and is influenced by many abiotic and biotic factors including soil pH, moisture content, temperature, nutritional level of soil and plants, microbial competition and predation (Marschner et al. 2004). Therefore, mixing PL or BS into soil may have changed the soil conditions, and as a result, influenced the efficacy of the selected PGPR strains.

4.5 Conclusions

In this study, PL had a greater influence than two other fertility sources, on corn plants, increasing plant biomass, both above- and below-ground, at all of the vegetative growth stages

evaluated (V4, V6, and VT). The selected PGPR mixtures improved some of the above- and below-ground plant growth parameters, with the greatest influence observed at V4 and V6 growth stages. However, these improvements did not show a consistent trend across the growth parameters evaluated regardless of fertility source. Applying the fertilizers sources at a 135 kg N ha^{-1} (optimal recommended N rate) may have masked the influence of PGPR on corn growth parameters. Future studies should be conducted to investigate the optimal N rate and source to improve the efficacy of PGPR on crop growth.

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Table 1. Physical and chemical properties of initial soil, poultry litter, and biosolid on a dry weight basis.

Property	Initial soil	Poultry litter	Biosolid
Bulk density (g cm^{-3})	1.30	-	-
pH (1:1 soil:water)	5.60	-	5.57
Moisture content (g 100 g^{-1})	13.52	18.5	40.9
Total C (g kg^{-1})	-	231.40	156.20
Total N (g kg^{-1})	0.01	29.20	16.90
C:N ratio	-	7.92	9.24
P (g kg^{-1})	0.02	19.70	8.60
K (g kg^{-1})	0.02	36.80	1.20
Ca (g kg^{-1})	0.13	34.70	99.60
Mg (g kg^{-1})	0.03	9.70	15.30
Na (g kg^{-1})	-	15.54	0.46
Cu (mg kg^{-1})	0.20	716	118
Fe (mg kg^{-1})	12.00	4132	12599
Mn (mg kg^{-1})	10.50	655	608
Zn (mg kg^{-1})	0.55	690	2003

†P, K, Ca, Mg, Na, Cu, Fe, Mn, and Zn values represent Mehlich extractable nutrient concentrations for soil and total nutrient concentrations for poultry litter.

Table 2. Bacteria species and strains present in the PGPR mixtures used in this study.

PGPR Mix	Original Strain†	Identification
1	JM-339	*Pseudomonas moraviensis*
	17A-3	*Bacillus amloliquefaciens*
	1PJ-32	*B. methylotrophicus*
	SE-34	*B. altitudinis*
2	2RA-17	*B. cereus*
	99-101	*B. amyloliquefaciens*
	33B-9	*B. mojavensis*
	IN-937a	*B. subtilis subsp. subtilis*
3	INR-7	*B. altutydinis*
	FZB-42	*B. amyloliquefaciens*
	SE-34	*B. altitudinis*
	SE-76	*B. altitudinis*
4	SE-52	*B. safensis*
	INR-7	*B. altitudinis*
	SE-56	*Lysinibacillus xylanilyticus*
	E-681	*Paenibacillluspeoriae*

†PGPR strains were selected by screening testing (IAA production, ammonia production, phosphate solubilization, siderophore production, germination test, and seedling test).

Table 3. PGPR effects on corn plant height, stem diameter, and leaf area as influenced by the different fertility sources during V4, V6, and VT growth stages at the HP location.

PGPR	Plant height (cm)			Stem diameter (mm)			Leaf area (cm^2)		
	V4	V6	VT	V4	V6	VT	V4	V6	VT
					IF treatments†				
Control	58.7 ± 3.4	88.2 ± 2.9	182 ± 13	5.9 ± 0.5	11.0 ± 0.9	14.5 ± 0.5	289 ± 35	904 ± 159	2370 ± 51
P1	57.3 ± 3.1	82.8 ± 7.9	189 ± 11	5.6 ± 0.6	13.3 ± 1.0	12.7 ± 0.4	295 ± 32	896 ± 150	2000 ± 161
P2	63.7 ± 5.0	89.5 ± 4.4	199 ± 11	6.8 ± 0.9	13.7 ± 1.3	13.0 ± 0.3	393 ± 82	1015 ± 117	2300 ± 251
P3	46.8 ± 3.4	78.3 ± 3.4	177 ± 13	5.6 ± 0.8	12.8 ± 0.6	12.2 ± 1.1	201 ± 31	932 ± 159	1978 ± 222
P4	57.7 ± 3.4	89.0 ± 2.1	198 ± 20	5.5 ± 0.5	13.2 ± 0.5	11.8 ± 1.0	270 ± 25	960 ± 69	2109 ± 161
					BS treatments				
Control	54.6 ± 3.4	78.9 ± 3.0	167 ± 15	5.6 ± 0.8	12.5 ± 0.7	13.3 ± 0.7	256 ± 36	808 ± 82	2253 ± 184
P1	54.2 ± 3.4	83.4 ± 4.0	175 ± 5.7	5.1 ± 0.3	11.5 ± 0.3	13.1 ± 0.9	279 ± 40	966 ± 88	2093 ± 89
P2	53.5 ± 2.0	86.0 ± 1.4	164 ± 15	5.5 ± 0.3	12.1 ± 0.6	12.4 ± 0.3	246 ± 18	912 ± 61	2104 ± 126
P3	57.9 ± 0.9	85.4 ± 3.9	160 ± 9.1	5.7 ± 0.9	11.9 ± 0.5	12.8 ± 0.7	255 ± 6.2	984 ± 79	2118 ± 170
P4	60.5 ± 6.1	77.3 ± 4.7	170 ± 15	6.3 ± 0.6	11.1 ± 1.2	12.9 ± 0.4	273 ± 45	738 ± 155	2311 ± 133
					PL treatments				
Control	77.4 ± 3.4	77.1 ± 2.5 b‡	176 ± 16	9.4 ± 0.7	16.1 ± 0.5	13.4 ± 0.5	556 ± 79	944 ± 136	2272 ± 138
P1	70.4 ± 2.6	80.3 ± 1.6 ab	183 ± 17	8.6 ± 0.5	17.1 ± 0.2	14.1 ± 0.6	543 ± 73	1231 ± 114	2288 ± 161
P2	72.1 ± 5.4	84.4 ± 1.5 a	191 ± 16	10.2 ± 1.1	18.3 ± 1.1	14.7 ± 0.7	499 ± 96	1015 ± 53	2271 ± 125
P3	67.2 ± 2.4	74.4 ± 2.4 b	191 ± 11	7.4 ± 0.8	16.0 ± 0.4	14.9 ± 0.6	411 ± 35	1020 ± 130	2034 ± 198
P4	78.5 ± 1.3	74.9 ± 2.4 b	207 ± 9.5	10.3 ± 0.8	16.1 ± 0.6	14.1 ± 0.5	600 ± 52	880 ± 100	2259 ± 167
					P > *F*				
IF treatments	0.0854	0.3779	0.7563	0.6242	0.3009	0.1305	0.0972	0.9686	0.6189
BS treatments	0.7087	0.3543	0.9511	0.6695	0.6854	0.8545	0.8293	0.3621	0.7735
PL treatments	0.1232	0.0203	0.6225	0.1114	0.0965	0.4415	0.4159	0.3138	0.7713
Fertility (N)	< 0.001	0.0092	0.0181	< 0.001	< 0.001	0.0011	< 0.001	0.1721	0.8002
PGPR	0.1555	0.1423	0.5731	0.1755	0.1565	0.6934	0.3454	0.3374	0.4448
N*PGPR	0.3858	0.2912	0.9588	0.5015	0.3538	0.1614	0.5448	0.7929	0.9348

†IF- inorganic fertilizer; BS- biosolid; PL- poultry litter.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

Table 4. PGPR effects on corn plant height, stem diameter, and leaf area as influenced by the different fertility sources during V4, V6, and VT growth stages at the PSRC location.

PGPR	Plant height (cm)			Stem diameter (mm)			Leaf area (cm^2)		
	V4	V6	VT	V4	V6	VT	V4	V6	VT
					IF treatments†				
Control	42.2 ± 3.6	58.1 ± 6.5	174 ± 8.5	3.7 ± 0.4	7.6 ± 0.5	8.6 ± 0.4	174 ± 20 c‡	516 ± 63 b	2409 ± 88
P1	46.3 ± 2.2	55.4 ± 3.6	169 ± 12	4.5 ± 0.4	7.6 ± 0.8	8.5 ± 0.5	263 ± 20 a	438 ± 50 b	2516 ± 99
P2	52.3 ± 3.1	61.5 ± 6.1	199 ± 8.5	4.5 ± 0.3	7.9 ± 0.4	8.3 ± 0.2	192 ± 15 bc	855 ± 112 a	2480 ± 104
P3	38.9 ± 4.3	55.8 ± 1.5	190 ± 12	5.0 ± 0.4	8.9 ± 0.6	8.6 ± 0.7	235 ± 13 ab	567 ± 74 b	2381 ± 59
P4	40.6 ± 5.2	53.9 ± 3.7	170 ± 18	4.4 ± 0.4	8.3 ± 0.5	8.1 ± 0.2	258 ± 47 abc	448 ± 51 b	2386 ± 63
					BS treatments				
Control	40.7 ± 4.1	59.0 ± 2.6	232 ± 15	5.5 ± 0.4	11.2 ± 0.7	10.1 ± 0.7	347 ± 48	1198 ± 302	2073 ± 252
P1	40.5 ± 1.4	65.1 ± 3.6	249 ± 2.7	5.2 ± 0.5	9.8 ± 0.7	9.5 ± 0.2	307 ± 51	987 ± 70	2474 ± 127
P2	43.3 ± 2.6	63.9 ± 6.3	246 ± 10	5.4 ± 0.5	12.4 ± 1.0	9.9 ± 0.3	327 ± 38	931 ± 60	2427 ± 112
P3	42.7 ± 3.6	59.0 ± 2.9	240 ± 15	4.8 ± 0.1	12.4 ± 1.8	10.0 ± 0.4	314 ± 49	1033 ± 118	2482 ± 188
P4	35.9 ±1.9	68.7 ± 3.0	244 ± 6.3	4.3 ± 0.2	11.7 ± 1.0	10.4 ± 0.1	325 ± 28	1035 ± 75	2373 ± 72
					PL treatments				
Control	47.5 ± 2.6	82.4 ± 2.6	267 ± 3.6	7.9 ± 0.6	13.5 ± 0.7	12.3 ± 0.5	450 ± 49	1418 ± 103	2727 ± 115
P1	45.9 ± 3.7	78.0 ± 3.1	256 ± 16	9.3 ± 0.1	14.5 ± 0.9	12.9 ± 0.2	540 ± 38	1656 ± 88	2765 ± 207
P2	50.4 ± 4.1	79.7 ± 5.6	277 ± 4.2	8.7 ± 0.7	15.2 ± 0.6	12.8 ± 0.2	584 ± 24	1744 ± 119	2586 ± 30
P3	51.2 ± 3.8	84.3 ± 1.9	251 ± 12	7.8 ± 0.5	14.2 ± 2.2	13.1 ± 1.0	498 ± 67	1491 ± 49	2620 ± 55
P4	49.3 ± 2.7	78.7 ± 5.2	264 ± 9.3	7.8 ± 0.8	14.1 ± 0.8	13.3 ± 0.5	456 ± 61	1622 ± 144	2677 ± 99
	P > F								
IF treatments	0.1755	0.8068	0.3609	0.1766	0.5086	0.9355	0.0079	0.0043	0.7291
BS treatments	0.5798	0.4224	0.8813	0.2448	0.4562	0.6944	0.8359	0.8047	0.4312
PL treatments	0.8166	0.7745	0.4414	0.2808	0.891	0.7486	0.3105	0.23	0.8128
Fertility (N)	0.0015	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0009
PGPR	0.2055	0.9684	0.4716	0.0858	0.4358	0.8829	0.2383	0.4652	0.6343
N*PGPR	0.4813	0.5861	0.5641	0.3138	0.8855	0.8567	0.6314	0.1404	0.5424

†IF, inorganic fertilizer; BS, biosolid; PL, poultry litter.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

Table 5. PGPR effects on corn root morphology as influenced by the different fertility sources during V4, V6, and VT growth stages at the HP location.

PGPR	Total length (cm)			Average diameter (mm)			Surface area (cm^2)			Total volume (cm^3)		
	V4	V6	VT	V4	V6	VT	V4	V6	VT	V4	V6	VT
						IF treatments†						
Control	2523 ± 173	6162 ± 440	19977 ± 2089	0.48 ± 0.01 b	0.63 ± 0.03	0.64 ± 0.02	382 ± 30	1227 ± 126	3941 ± 286	4.6 ± 0.4 ab	19.6 ± 2.7	62.6 ± 3.2
P1	2716 ± 188	5445 ± 766	19129 ± 1610	0.47 ± 0.02 b	0.61 ± 0.03	0.62 ± 0.02	398 ± 21	1068 ± 181	3677 ± 276	4.7 ± 0.3 ab	16.8 ± 3.4	56.5 ± 4.1
P2	2471 ± 246	5685 ± 838	17690 ± 1469	0.55 ± 0.04 a	0.69 ± 0.03	0.65 ± 0.02	434 ± 60	1252 ± 213	3640 ± 375	6.2 ± 1.2 a	22.1 ± 4.4	59.8 ± 7.5
P3	2032 ± 241	5656 ± 761	15467 ± 2428	0.48 ± 0.03 b	0.63 ± 0.04	0.63 ± 0.02	299 ± 28	1146 ± 194	3047 ± 482	3.5 ± 0.3 b	18.7 ± 3.8	48.1 ± 7.9
P4	2242 ± 214	5905 ± 204	20063 ± 3656	0.44 ± 0.01 b	0.66 ± 0.02	0.59 ± 0.02	311 ± 33	1229 ± 51	3745 ± 703	3.5 ± 0.4 b	20.4 ± 1.2	56.2 ± 11.3
						BS treatments						
Control	2562 ± 284	5815 ± 496 a‡	15079 ± 727	0.52 ± 0.03	0.65 ± 0.01	0.60 ± 0.02	427 ± 60	1183 ± 91	2821 ± 109	5.7 ± 1.0	19.2 ± 1.4	42.4 ± 2.2
P1	2432 ± 207	5811 ± 252 a	16530 ± 750	0.54 ± 0.01	0.65 ± 0.02	0.60 ± 0.02	413 ± 40	1188 ± 80	3111 ± 187	5.6 ± 0.6	19.4 ± 1.8	47.1 ± 4.3
P2	2236 ± 112	6523 ± 244 a	15354 ± 833	0.51 ± 0.01	0.65 ± 0.02	0.60 ± 0.02	355 ± 17	1332 ± 57	2873 ± 147	4.5 ± 0.3	21.7 ± 1.4	43.2 ± 2.7
P3	2624 ± 264	5995 ± 301 a	15136 ± 1410	0.55 ± 0.03	0.68 ± 0.03	0.61 ± 0.01	449 ± 23	1284 ± 94	2872 ± 243	6.1 ± 0.02	22.0 ± 2.3	43.6 ± 3.5
P4	2721 ± 176	4454 ± 430 b	13864 ± 1767	0.53 ± 0.03	0.65 ± 0.02	0.65 ± 0.03	458 ± 42	988 ± 113	2759 ± 270	6.2 ± 0.8	16.1 ± 2.1	44.0 ± 2.9
						PL treatments						
Control	2849 ± 152	5979 ± 393	17558 ± 1383	0.62 ± 0.02	0.74 ± 0.04	0.62 ± 0.02	561 ± 47	1397 ± 150	3424 ± 236	8.8 ± 1.1	26.3 ± 4.3	53.4 ± 3.7
P1	2791 ± 134	6742 ± 161	20085 ± 1650	0.65 ± 0.04	0.71 ± 0.04	0.61 ± 0.02	569 ± 45	1510 ± 75	3831 ± 295	9.4 ± 1.3	27.2 ± 2.7	58.6 ± 4.8
P2	3300 ± 167	6637 ± 218	19672 ± 1288	0.61 ± 0.05	0.77 ± 0.06	0.60 ± 0.02	632 ± 71	1605 ± 90	3696 ± 235	9.9 ± 1.9	31.6 ± 4.2	55.9 ± 4.6
P3	2895 ± 244	6150 ± 321	20689 ± 588	0.60 ± 0.05	0.74 ± 0.06	0.59 ± 0.01	540 ± 38	1423 ± 132	3802 ± 86	8.2 ± 1.0	27.0 ± 4.7	55.9 ± 1.3
P4	3355 ± 143	6356 ± 301	19998 ± 858	0.65 ± 0.04	0.74 ± 0.02	0.62 ± 0.01	684 ± 61	1486 ± 89	3873 ± 195	11.3 ± 1.7	27.7 ± 2.2	60.0 ± 3.7
						P > *F*						
IF treatments	0.2326	0.9478	0.6302	0.038	0.4511	0.2626	0.0765	0.9244	0.7012	0.037	0.8301	0.7066
BS treatments	0.5355	0.0136	0.6849	0.7582	0.7684	0.4555	0.4958	0.1048	0.8145	0.4956	0.2003	0.8739
PL treatments	0.0836	0.3821	0.4431	0.8856	0.94	0.5285	0.3301	0.7171	0.6126	0.6112	0.8748	0.7615
Fertility (N)	< 0.001	0.0591	0.0004	< 0.001	< 0.001	0.2957	< 0.001	0.0004	0.0002	< 0.001	< 0.001	0.0002
PGPR	0.7228	0.4906	0.8699	0.9701	0.5857	0.8365	0.7205	0.556	0.8368	0.8028	0.5055	0.804
N*PGPR	0.0952	0.3025	0.5322	0.3234	0.9586	0.1994	0.1379	0.806	0.7385	0.3283	0.9585	0.82

†IF, inorganic fertilizer; BS, biosolid; PL, poultry litter.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

Table 6. PGPR effects on corn root morphology as influenced by the different fertility sources during V4, V6, and VT growth stages at the PSRC location.

PGPR	Total length (cm)			Average diameter (mm)			Surface area (cm^2)			Total volume (cm^3)		
	V4	V6	VT	V4	V6	VT	V4	V6	VT	V4	V6	VT
						IF treatments†						
Control	1505 ± 301	4117 ± 235	6340 ± 375	0.35 ± 0.01	0.41 ± 0.02	0.59 ± 0.03	169 ± 36 bc	533 ± 48	1179 ± 108	1.5 ± 0.3 b	5.5 ± 0.7	17.7 ± 2.5
P1	2428 ± 244	4146 ± 473	6929 ± 408	0.37 ± 0.01	0.41 ± 0.01	0.56 ± 0.01	286 ± 30 a	533 ± 64	1223 ± 85	2.7 ± 0.3 a	5.5 ± 0.7	17.2 ± 1.4
P2	1460 ± 278	3339 ± 256	6351 ± 260	0.36 ± 0.02	0.43 ± 0.01	0.62 ± 0.04	157 ± 27 c	458 ± 48	1234 ± 47	1.4 ± 0.2 c	5.0 ± 0.7	19.4 ± 1.8
P3	2157 ± 324	4610 ± 270	6550 ± 503	0.38 ± 0.01	0.46 ± 0.02	0.61 ± 0.05	258 ± 44 ab	667 ± 58	1246 ± 79	2.5 ± 0.5 ab	7.7 ± 0.9	19.3 ± 2.4
P4	1853 ± 162	3625 ± 326	6506 ± 157	0.36 ± 0.01	0.41 ± 0.01	0.58 ± 0.02	208 ± 20 abc	464 ± 47	1181 ± 45	1.9 ± 0.2 abc	4.7 ± 0.5	17.1 ± 1.1
						BS treatments						
Control	2010 ± 361	3469 ± 433	6997 ± 330	0.36 ± 0.01	0.45 ± 0.01 ab‡	0.71 ± 0.03	234 ± 49	497 ± 69	1550 ± 91	2.2 ± 0.5	5.7 ± 0.9	27.6 ± 2.7 b
P1	1615 ± 360	3571 ± 286	7084 ± 377	0.39 ± 0.01	0.41 ± 0.02 b	0.72 ± 0.02	202 ± 47	469 ± 57	1607 ± 97	2.0 ± 0.5	4.9 ± 0.8	29.1 ± 2.3 b
P2	1922 ± 216	3333 ± 346	9832 ± 1935	0.38 ± 0.01	0.42 ± 0.01 b	0.68 ± 0.06	229 ± 25	440 ± 51	1982 ± 225	2.2 ± 0.2	4.6 ± 0.6	32.9 ± 1.7 ab
P3	1885 ± 354	3394 ± 279	7219 ± 331	0.38 ± 0.01	0.48 ± 0.02 a	0.75 ± 0.02	225 ± 41	516 ± 56	1689 ± 83	2.1 ± 0.4	6.3 ± 0.8	31.6 ± 2.0 b
P4	1641 ± 184	3705 ± 96	7866 ± 1341	0.39 ± 0.01	0.42 ± 0.01 b	0.86 ± 0.10	201 ± 22	486 ± 28	1991 ± 136	2.0 ± 0.2	5.1 ± 0.5	42.9 ± 6.3 a
						PL treatments						
Control	1871 ± 181	3874 ± 378	17006 ± 626	0.38 ± 0.01	0.41 ± 0.01	0.49 ± 0.01	228 ± 28	506 ± 57	2601 ± 131	2.21 ± 0.34	5.3 ± 0.7	31.8 ± 2.3
P1	2141 ± 46	3941 ± 583	16555 ± 527	0.39 ± 0.02	0.45 ± 0.01	0.53 ± 0.01	264 ± 9.3	556 ± 81	2712 ± 80	2.60 ± 0.19	6.3 ± 0.9	35.7 ± 1.1
P2	2036 ± 321	5318 ± 295	17395 ± 562	0.39 ± 0.01	0.46 ± 0.03	0.51 ± 0.03	250 ± 39	766 ± 80	2773 ± 201	2.44 ± 0.39	8.9 ± 1.5	35.7 ± 4.7
P3	1879 ± 459	5567 ± 572	14433 ± 2219	0.40 ± 0.02	0.41 ± 0.02	0.48 ± 0.05	234 ± 56	733 ± 103	2402 ± 360	2.33 ± 0.54	7.7 ± 1.4	32.0 ± 4.8
P4	1820 ± 181	4927 ± 378	16194 ± 555	0.42 ± 0.01	0.42 ± 0.01	0.54 ± 0.02	242 ± 53	643 ± 87	2734 ± 106	2.56 ± 0.62	6.7 ± 0.9	37.1 ± 2.3
						P > *F*						
IF treatments	0.0864	0.0907	0.7807	0.5855	0.1203	0.6593	0.0496	0.0743	0.9537	0.0384	0.0574	0.8387
BS treatments	0.8804	0.9377	0.3477	0.1608	0.019	0.2423	0.9726	0.8917	0.0946	0.9968	0.5863	0.043
PL treatments	0.9417	0.1087	0.4034	0.4495	0.1881	0.5295	0.974	0.17	0.7023	0.9674	0.2226	0.7407
Fertility (N)	0.8217	< 0.001	< 0.001	< 0.0001	0.4309	< 0.001	0.4097	0.0011	< 0.001	0.1435	0.0126	< 0.001
PGPR	0.2825	0.2392	0.2757	0.0614	0.0649	0.3296	0.1952	0.1298	0.2798	0.1495	0.0843	0.0973
N*PGPR	0.7873	0.0647	0.4907	0.6159	0.02	0.2766	0.7739	0.1993	0.5936	0.7575	0.2508	0.2336

†IF, inorganic fertilizer; BS, biosolid; PL, poultry litter.

‡Means and standard errors followed by the same letter or with no letter assignment within a column are not significantly different at $P < 0.05$.

Table 7. PGPR effects on corn total C and N concentrations as influenced by the different fertility sources after harvest at the VT stage at the HP location.

PGPR	Total Carbon (%)			Total Nitrogen (%)		
	Root	Stem	Leaf	Root	Stem	Leaf
			IF treatments†			
Control	45.85 ± 0.13	45.30 ± 0.14	45.01 ± 0.08	1.36 ± 0.07	1.15 ± 0.08	2.43 ± 0.08
P1	45.62 ± 0.16	45.10 ± 0.09	45.92 ± 0.22	1.72 ± 0.04	1.24 ± 0.06	2.58 ± 0.06
P2	46.05 ± 0.07	45.44 ± 0.08	45.89 ± 0.19	1.56 ± 0.05	1.18 ± 0.06	2.55 ± 0.15
P3	44.96 ± 0.49	45.25 ± 0.33	45.92 ± 0.11	1.66 ± 0.16	1.45 ± 0.15	2.71 ± 0.24
P4	45.51 ± 0.48	44.88 ± 0.50	45.82 ± 0.24	1.38 ± 0.16	1.29 ± 0.23	2.66 ± 0.23
			BS treatments			
Control	46.07 ± 0.41	45.46 ± 0.21	45.29 ± 0.09	0.99 ± 0.10	0.86 ± 0.10	1.74 ± 0.24
P1	46.35 ± 0.14	45.59 ± 0.07	45.37 ± 0.13	0.95 ± 0.04	0.67 ± 0.05	1.43 ± 0.11
P2	46.19 ± 0.14	45.52 ± 0.08	45.23 ± 0.15	1.01 ± 0.07	0.73 ± 0.07	1.62 ± 0.13
P3	46.54 ± 0.11	45.44 ± 0.13	45.57 ± 0.10	0.94 ± 0.03	0.70 ± 0.03	1.54 ± 0.12
P4	46.37 ± 0.16	45.71 ± 0.10	45.61 ± 0.10	1.01 ± 0.07	0.81 ± 0.04	1.63 ± 0.11
			PL treatments			
Control	45.34 ± 0.11	44.54 ± 0.11	45.11 ± 0.08	0.84 ± 0.03	0.68 ± 0.04	1.73 ± 0.07
P1	44.79 ± 0.27	44.36 ± 0.10	44.73 ± 0.36	0.95 ± 0.09	0.74 ± 0.12	1.47 ± 0.23
P2	45.29 ± 0.14	44.60 ± 0.17	45.23 ± 0.05	0.82 ± 0.03	0.61 ± 0.03	1.47 ± 0.08
P3	45.17 ± 0.17	44.51 ± 0.17	45.25 ± 0.02	0.83 ± 0.05	0.68 ± 0.05	1.47 ± 0.13
P4	45.42 ± 0.10	44.75 ± 0.04	44.98 ± 0.08	0.83 ± 0.03	0.64 ± 0.04	1.18 ± 0.05
	P > F					
IF treatments	0.2029	0.6802	0.9648	0.1096	0.5397	0.7898
BS treatments	0.6385	0.6079	0.1032	0.9175	0.2663	0.7230
PL treatments	0.1157	0.3605	0.2255	0.3660	0.6840	0.4531
Fertility (N)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
PGPR	0.5665	0.8808	0.5028	0.2272	0.7552	0.9511
N*PGPR	0.1007	0.5588	0.3605	0.1755	0.4118	0.6020

†IF, inorganic fertilizer; BS, biosolid; PL, poultry litter.

Table 8. PGPR effects on corn total C and N concentrations as influenced by the different fertility sources after harvest at the VT stage at the PSRC location.

PGPR	Total Carbon (%)			Total Nitrogen (%)		
	Root	Stem	Leaf	Root	Stem	Leaf
			IF treatments†			
Control	44.11 ± 0.29	43.44 ± 0.28	45.96 ± 0.18	2.09 ± 0.10	1.65 ± 0.07	3.39 ± 0.07
P1	44.32 ± 0.34	43.58 ± 0.28	46.02 ± 0.09	1.93 ± 0.12	1.63 ± 0.05	3.38 ± 0.11
P2	44.36 ± 0.09	43.83 ± 0.23	46.00 ± 0.16	2.03 ± 0.03	1.54 ± 0.06	3.42 ± 0.05
P3	44.30 ± 0.23	43.69 ± 0.23	45.92 ± 0.15	2.01 ± 0.05	1.60 ± 0.08	3.46 ± 0.05
P4	43.96 ± 0.21	43.47 ± 0.37	45.86 ± 0.11	2.04 ± 0.06	1.66 ± 0.10	3.41 ± 0.07
			BS treatments			
Control	44.94 ± 0.25	44.82 ± 0.36	45.06 ± 0.14	1.45 ± 0.08	1.19 ± 0.13	2.80 ± 0.15
P1	44.99 ± 0.08	45.02 ± 0.05	45.94 ± 0.06	1.41 ± 0.04	1.19 ± 0.03	2.74 ± 0.07
P2	45.05 ± 0.13	45.10 ± 0.08	45.98 ± 0.05	1.33 ± 0.05	1.11 ± 0.04	2.62 ± 0.10
P3	45.53 ± 0.09	45.01 ± 0.05	46.01 ± 0.02	1.36 ± 0.05	1.05 ± 0.05	2.57 ± 0.10
P4	45.27 ± 0.21	45.14 ± 0.09	45.97 ± 0.09	1.30 ± 0.04	0.98 ± 0.04	2.42 ± 0.11
			PL treatments			
Control	45.40 ± 0.07	44.41 ± 0.10	45.29 ± 0.08	0.92 ± 0.04	0.71 ± 0.02	1.74 ± 0.10
P1	45.20 ± 0.17	44.45 ± 0.06	45.36 ± 0.05	0.87 ± 0.06	0.68 ± 0.03	1.68 ± 0.05
P2	45.40 ± 0.04	44.44 ± 0.17	45.34 ± 0.01	0.94 ± 0.02	0.70 ± 0.08	1.64 ± 0.06
P3	45.43 ± 0.16	44.43 ± 0.10	45.52 ± 0.10	1.00 ± 0.07	0.72 ± 0.08	1.87 ± 0.16
P4	45.65 ± 0.08	44.42 ± 0.08	45.48 ± 0.07	0.87 ± 0.02	0.70 ± 0.05	1.64 ± 0.04
	P > F					
IF treatments	0.7519	0.8465	0.6955	0.7045	0.8038	0.9323
BS treatments	0.1154	0.7031	0.8921	0.3064	0.1598	0.1577
PL treatments	0.1489	0.9337	0.1299	0.2880	0.9865	0.3680
Fertility (N)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
PGPR	0.3940	0.7173	0.7540	0.3853	0.5856	0.2524
N*PGPR	0.3458	0.9625	0.5591	0.6071	0.5290	0.3017

†IF, inorganic fertilizer; BS, biosolid; PL, poultry litter.

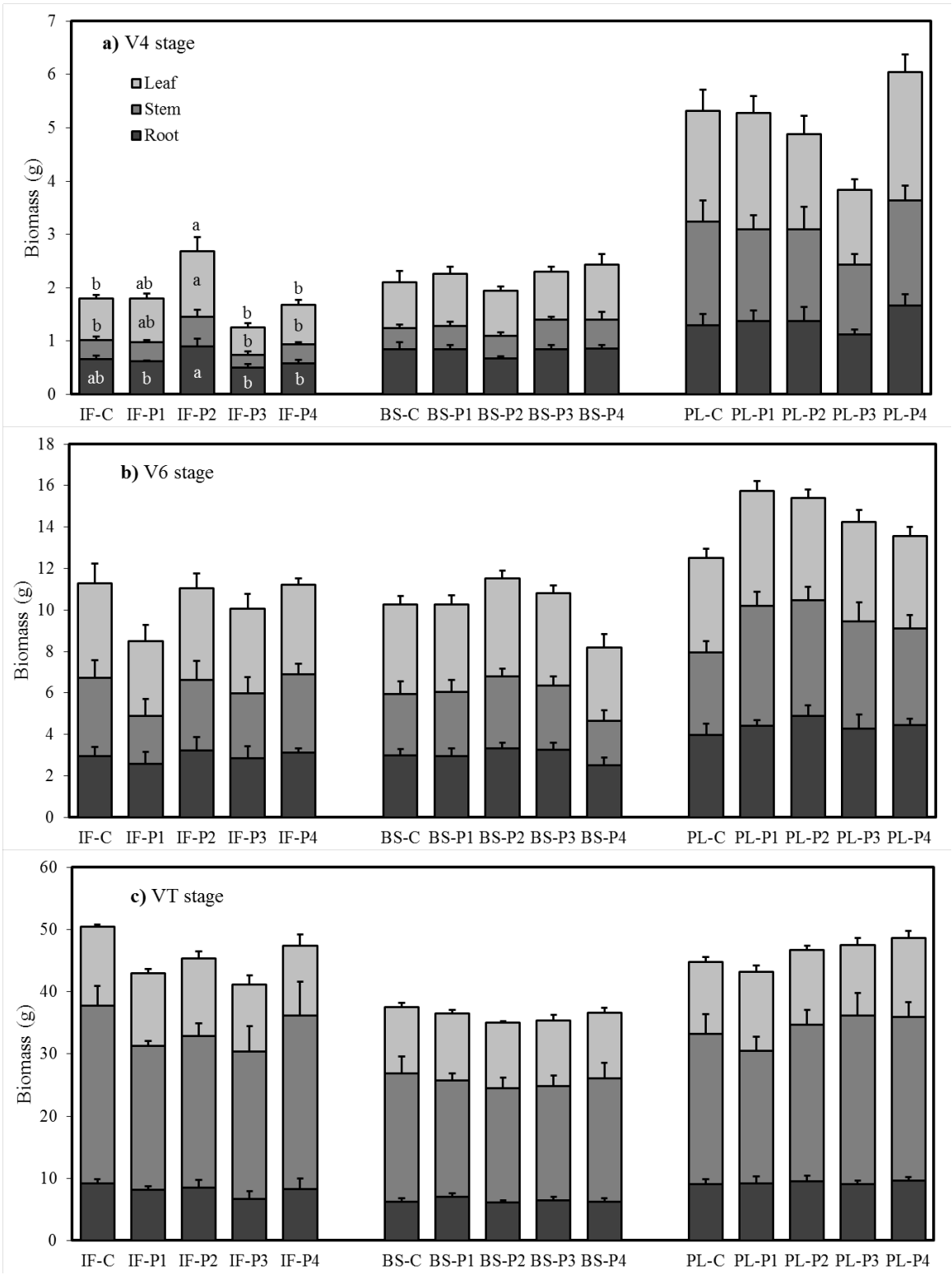

Fig. 1. Corn biomass (dry weight basis) from the glasshouse container study at the HP location for PGPR inoculation as influenced by the different fertility sources during V4, V6, and VT growth stages. Data represent means and standard errors of replicates. The bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$ for multiple comparisons under the same fertilizer sources.

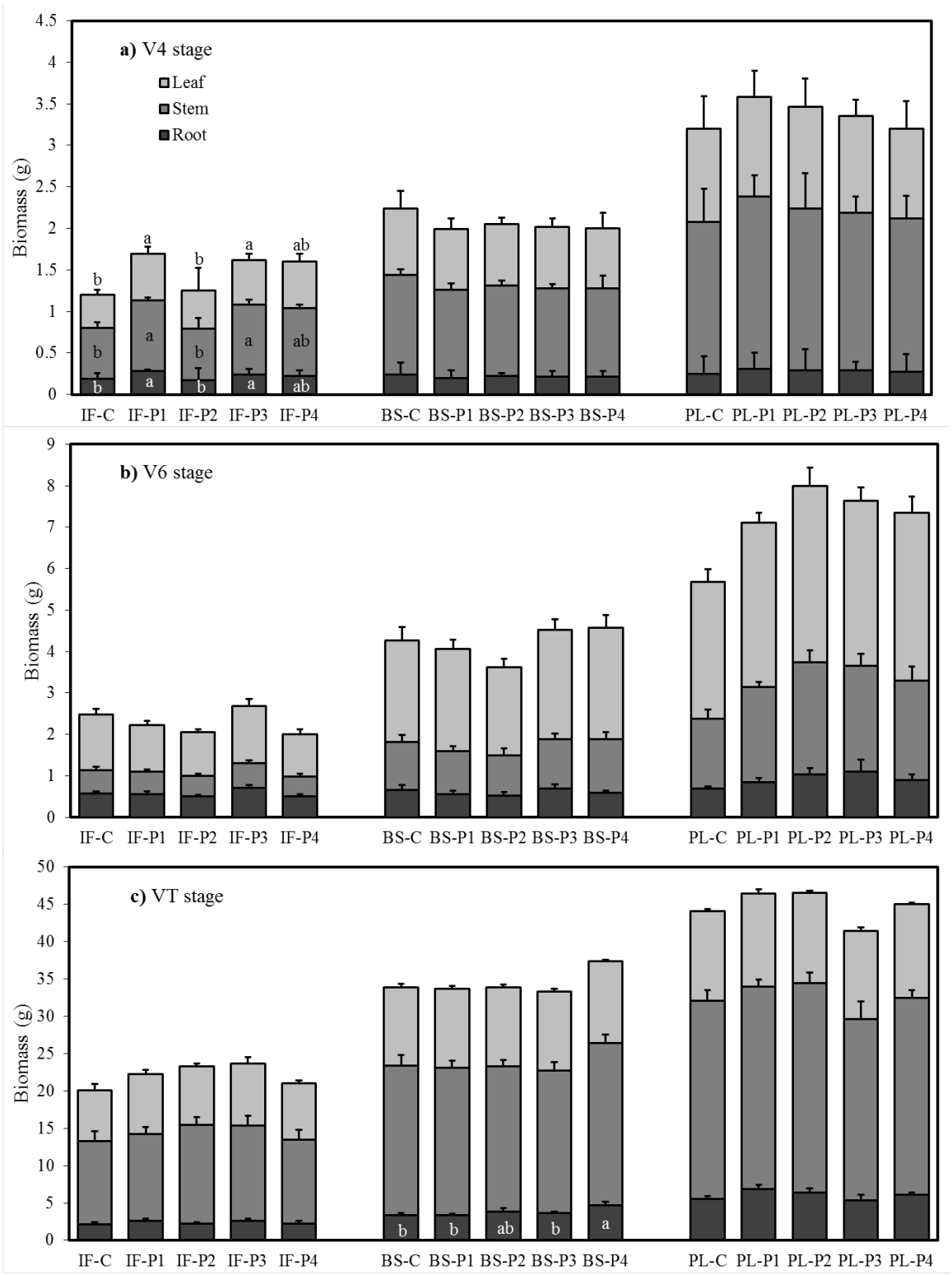

Fig. 2. Corn biomass (dry weight basis) from the glasshouse container study at the PSRC location for PGPR inoculation as influenced by the different fertility sources during V4, V6, and VT growth stages. Data represent means and standard errors of replicates. The bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$ for multiple comparisons under the same fertilizer sources.

5. Effect of PGPR on Corn Growth at Various Nitrogen Rates

5.1 Abstract

Plant growth-promoting rhizobacteria (PGPR) are capable of aggressively colonizing plants roots and promoting plant growth by producing and secreting various chemical regulators around the rhizosphere. With the recent interest in sustainable agriculture, an increasing number of researchers are investigating ways to maximize the efficiency of PGPR use while reducing chemical fertilizer inputs needed for crop production. Therefore, a glasshouse study was conducted to evaluate the impact of PGPR inoculants on biomass production and N content of maize (*Zea mays* L.) under different N levels. Treatments included three PGPR inoculants (2 PGPR strain mixtures and 1 control without PGPR) and five N application levels at 0, 25, 50, 75, and 100% of the recommended N rate (135 kg N ha^{-1}). Results show that inoculation of PGPR significantly increased maize growth parameters (e.g., plant height, stem diameter, leaf area, and root morphology) compared to no PGPR application under the same N levels at the V6 growth stage, but little difference was 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. We concluded that PGPR inoculant mixtures could reduce inorganic N fertilization without affecting maize plant growth parameters. Future research is needed under field conditions to determine if these PGPR inoculants could be integrated as a bio-fertilizer in crop production nutrient management strategies.

5.2 Introduction

Commercial fertilizers, especially N sources, are necessary for maintaining global crop production and fulfilling food requirements for a rapidly growing world population with limited land resources (Stewart et al. 2005; Roberts 2009; Ribaudo et al. 2011; Marschner 2012). It has

been reported that more than 11.7 million tons of N fertilizer was applied to North American soils in 2014 (Apodaca 2016). This number is expecting to increase in the coming years because inorganic N has become an indispensable commodity. For example, Stewart et al. (2005) evaluated several long-term studies to determine the effect of eliminating N fertilizer, and predicted that corn and cotton (*Gossypium hirsutum* L.) yield would decline by 41 and 37% without N fertilizer, respectively. Optimal crop yields also depends upon the N use efficiency (NUE) of crops. Generally, NUE is very low (~ 33%; Raun and Johnson 1999) due to various soil processes and environmental factors (Adesemoye et al. 2010; Agostini et al. 2010). For example, over half of N applied can be lost from agricultural systems as gaseous loss (N_2, nitrous oxide, NH_3 etc.), runoff (NO_3), or leaching (NO_3) into groundwater (Vitousek et al. 1997; Tilman 1998). This poor utilization efficiency demands more effective management practices for improving NUE.

Microorganisms that have exhibited plant growth promoting abilities may be worth evaluating as a prospective tool to improve fertilizer use efficiency (Gadagi et al. 2004, Adesemoye et al. 2008; Adesemoye and Kloepper 2009; Cong et al. 2009; Ahmad et al. 2017). Plant growth-promoting rhizobacteria (PGPR) have been identified as a group of free living microbes that live on or around the roots (Kloepper; 1989) having the ability to stimulate plant growth and enhance root development and architecture (Kloepper et al. 1991; Canbolat et al. 2006; Adesemoye et al. 2009; Figueiredo et al. 2010). Kumar et al. (2009) indicated that applying *Pseudomonas aeruginosa* at half the recommended fertilizer rate resulted in growth that was equivalent to treatments at the full fertilizer rate for sesame (*Sesamum indicum* L.), while the oil yield increased 33.3%, and protein yield increased 47.5%, as compared to the full fertilizer rate. Adesemoye et al. (2009) found that supplementing 75% of the recommended fertilizer with

a mixture of *Bacillus* spp. and arbuscular mycorrhiza fungus (AMF) produced tomato's (*Solanum lycopersicum*) growth, yield, N and P uptake 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 (Shahzad et al. 2013) and inoculating *Rhodopseudomonas palustris* to soil with 50% of recommended chemical fertilizer rate for Chinese cabbage (*Brassica rapa chinesis*; Wong et al. 2014) had the same plant growth potential as the full fertilizer rate.

Among the PGPR microorganisms, *Bacillus* is one genera that has been widely used to enhance plant growth and suppress plant diseases (Kumar et al. 2011), mainly due to their wide metabolic capabilities allowing them to play important roles in soil ecosystem functions and processes, such as soil carbon (C), nitrogen (N), and sulfur (S) cycling, as well as the transformation of other soil nutrients (Mandic-Mulec and Prosser 2011). Huang et al. (2015) isolated four *Bacillus* strains from rainforest soils and reported that these strains increased plant height and shoot biomass of *Arabidopsis*, corn, and tomatoes under greenhouse conditions. Likewise, the selected *Bacillus* strains enhanced plant heights and plant fresh weights of tomatoes in both nutrient-poor soils and soils which received 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 (Wani and Khan 2010). Meng et al. (2016) 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 has also been observed with canola (*Brassica napus* L.,

de Freitas et al. 1997), corn (Kuan et al. 2016), soybean (*Glycine max*, Bullied et al. 2002), sugar beet (*Beta vulgaris*, Çakmakçi et al. 2006), and wheat (*Triticum aestivum* L., de Freitas 2000).

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. Thus, growth promotion was limited when using single-strain inoculations (Lucy et al. 2004). For example, *B. velezensis* inoculation increased dry leaf weight, but not root weight for the vegetative crops evaluated (Meng et al. 2016). de Freitas et al. (1997) reported that *Bacillus* spp. had no effect on canola growth when rock phosphate was applied, while increased seed yield, but not P uptake, was observed when triple superphosphate was applied. Similarly, de Freitas (2000) noticed 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 evaluated the use of PGPR strain mixtures for improving plant growth, and reported more consistent positive effects (Belimov et al. 1995; Ryu et al. 2007; Jarak et al. 2012). In addition, some studies have suggested that PGPR are more effective under limited nutrient conditions (Egamberdiyeva 2007; Shaharoona et al. 2008; Huang et al. 2015). 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 (Shaharoona et al. 2008). 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 application levels, (2) investigate whether the use PGPR mixtures

will allow for a reduction in the amount of inorganic N fertilizer needed for attaining corn plant growth and nutrient uptake levels equivalent to those at the recommended N fertilizer rate, and (3) investigate the optimal N rate for stimulating PGPR effects on corn.

5.3 Materials and Methods

A glasshouse container study was conducted at Auburn University's Horticulture-Paterson Greenhouse Complex (HP) in Auburn, AL. This study consisted of two separate experiments being conducted with the same treatments. The first experiment was conducted from March to May and second experiment from April to June of 2017 in the same glasshouse. The soil used for this study was a Kalmia sandy loam (fine-loamy over sandy, siliceous, semiactive, thermic Typic Hapludults) collected from the Alabama Agricultural Experiment Station's 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 submitted to the Auburn University's Soil Testing Laboratory and analyzed for nutrient concentrations according to procedures described by Hue and Evans (1986). Briefly, the soil had a pH of 5.50, 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 been shown to have positive effects on plant growth and were selected from previous screening experiments. The bacterial mixtures were prepared by mixing each strain's spore or cell suspension, which was previously quantified by plating the suspension on tryptic soy agar (TSA) and incubating for 48 h at 25 ºC, in equal concentrations. A bacterial mixture of 1 x 10^6 spore ml^{-1} was used for this study. The N rate treatments consisted of applying 0, 25, 50, 75, and 100% of the rate recommended by Alabama Cooperative Extension System for corn on a Coastal Plain soil, which is 135 kg N ha^{-1} (Mitchell and Huluka 2012). 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 x 23 cm at the top, and tapered to 18 x 18 cm at the base. The containers were filled with 12.5 kg of soil and watered to saturation based on five extra containers designated for this purpose. 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 glasshouses was maintained at 26 ± 2 °C during the day and 20 ± 3 °C at night. To minimize micro-environmental variation among treatments, containers were rotated weekly at random, by treatment.

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 plant's base to the top of the newest fully developed leaf. Stem diameter was determined using high-precision digital calipers. 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 ºC) 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 10000 professional scanner (Seiko Epson Corp., Shinjuku, Tokyo, Japan). Each individual root system was evenly spread apart placed in a water bath on a transparent tray (30 x 40 cm width) and imaged at a resolution of 157.5 dots per cm as described by Bauhus and Messier (1999) and Costa et al. (2000). The following root characteristics were determined: total root length (cm), root surface area (cm^2), root volume (cm^3), and average root diameter (mm). Plant total N was determined on the dried shoot and root tissues. Ground plant tissue (0.2 mm mesh) of leaves, stems, and roots harvested at the VT stage was analyzed for N using the dry combustion method (LECO FP-528 Nitrogen/Protein Analyzer, LECO Corp., Saint Joseph, MI, USA).

An analysis of variance (ANOVA), using a general linear model (GLM) of SAS 9.4 (SAS Institute Inc. 2013), 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.

5.4 Results and Discussion

5.4.1 Plant Growth Parameter

The N levels significantly affected the corn vegetative growth evaluated from the V4 to VT stages (Tables 2 and 3). Plant height can influence the number leaves per plant and potentially affect corn yield (Akintoye 1996). There were no significant differences or clear tendencies observed among the N levels evaluated on plant height at the V4 and VT stages, regardless of whether it was the first or second experiment being conducted. Plant height increased with increasing N rate during the first experiment (HP1). On average, 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. Our results were consistent to previous studies, which showed that the tallest plants were observed with the application of approximately 70% of the recommended N rate (Lustosa Filho et al. 2014; Marini et al. 2015). Arnon (1975) indicated that shorter plants resulting from low N availability may be associated with delayed cell division at the growing points. Plant height is influenced by soil nutrient content, soil moisture, temperature, sunlight duration, and other environmental factors. Soil moisture and temperature were suitable for plant growth under the glasshouse conditions of this study, so all plants had normal plant height regardless of N levels. Significant effects of microbial inoculations 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 observed between PGPR inoculants and non-inoculated treatments at the V4 and VT stages for both experiments (Tables 2 and 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 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. 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.

Stem diameter was significantly affected by soil N level, especially during the latter vegetative growth stages (Tables 2 and 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 increases 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 50% of the recommended N rate had the strongest 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 Dourado Neto (2000) reported that stronger stems were directly related to increased productivity since stems are involved in the storage of soluble solids, which may subsequently be used in the formation of seeds. PGPR inoculations 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 (Tables 2 and 3). However, PGPR mixture 1 tended to increased stem diameter for the no N fertilizer (N0P1) treatment at the V4 stage in HP1, which was significantly greater than that of the N100P0 treatment ($P = 0.0467$).

There were no significant effects of soil N level on leaf area at the V4 and VT stages for both experimental times (Tables 2 and 3). However, average leaf area at the recommended N rate was significantly larger than those 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 (Tables 2 and 3), while PGPR inoculations tended to increase plant leaf area 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 (Tables 2 and 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 3). Significant differences were observed between PGPR inoculants at the V6 and VT stages in HP1. 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 increases of 4.5 and 12.3%, respectively. Moreover, PGPR mixture 1 with no N application (N0P1) had the greatest leaf area compared to other treatments, and the leaf area was significantly greater than that of the N100P0 treatment ($P = 0.0183$) at the V6 stage in HP2. 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

mixture 2 when 50% of the recommended N rate ($P = 0.0322$) was compared to the no-PGPR control at the same N rate.

The uptake of N by corn is low during early development and increases as it nears tasseling (Amin 2011), which means that N generally has minimal effects on plant growth during the seedling stages. This explains why little differences were observed for plant growth parameters at the V4 stage. Moreover, another important factor that may affect seedling growth is the emergence day, as earlier emergence could lead to taller plants, greater stem diameters and leaf areas. Since temperature and sunlight duration were increasing from March to June, 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 thrive from greater N uptake from soil. 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 (Jarak et al. 2012; Dicko and Verma 2014; Calvo et al. 2017), strengthen plant stems (Çakmakçi et al. 2006; Jarak et al. 2012; Sengupta et al. 2015), and enhance the number of leaves and leaf area (Çakmakçi et al. 2006; Nezarat and Gholami 2009; Sengupta et al. 2015). However, once corn growth reached the tasseling stage, the nutrients provided through microbial activities (e.g., N fixation and P solubilization by PGPR) could not satisfy the high nutrient requirements of plants, and plant growth mainly relies on the applied fertilizer. In this study, PGPR showed more positive effects

under low N soils than in soil with high rates of N fertilization. Consistently, several studies have demonstrated that when soil nutrient levels are high, PGPR's efficacy to improve plant growth is low (Adesemoye et al. 2008; Carlier et al. 2008; Zabihi et al. 2010). One possible reason is that the production of ethylene under low levels of nutrients could be catabolized by ACC deaminase, produced by PGPR, to NH_3 and α-ketobutyrate (Miransari 2011). Also, in nutrient-rich soil, plants could obtain enough N from soil by their own root absorption, so it is not necessary to supply C for rhizobacteria to obtain more N for the roots.

5.4.2 Root Morphology

Plant roots tended to increase with increasing rates of N during both experiments (Tables 4 and 5), but significant differences were only observed for some of the root morphological parameters at the different growth stages. Applying 75% of recommended N rate resulted in greater average root diameter than the no N control at the V4 stage for HP1, with an average increase of 8.7%. Nitrogen application significantly improved root morphological parameters (average root diameter, root surface area, and total root volume) at the VT stage regardless of N rate ($P < 0.01$ for 25 – 100 % of recommended N rate) when compared to the unfertilized N control in HP1. When N levels reached to half of the recommended N rate, plant root average diameter, surface area, and total volume were equivalent to those of the 100% recommended N rate. The root morphology can be altered by soil N levels or N fertilization (Passioura and Wetselaar 1972; Maizlish et al. 1980; Bonifas and Lindquist 2009). Maizlish et al. (1980) reported that increasing N supply can lead to an increase in root length, the number of primary roots, and the elongation rate of first order laterals. However, greater root growth with no N fertilizer application in our study could be a result of increasing the amount of biomass allocated

to roots (Robinson 1986; Hilbert 1990; Bonifas et al. 2005) and also could be attributed to a larger root system through growing longer primary roots (Wang et al. 2005).

The influence of PGPR inoculants on root morphological parameters varied with N level and growth stage (Tables 4 and 5). Application of PGPR showed positive effects on root growth when low N rates were applied at the early growth stages in HP1. At the V4 stage, PGPR mixture 2 significantly increased average root diameter by 5.6% compared to the no-PGPR control in HP1. A significant N and PGPR interaction was observed for total root length at the V6 and VT stages in HP2, showing that PGPR inoculants had a positive effect on total root length at relative high N levels. 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, respectively, 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. These results indicate that the selected PGPR strains in this experiment could promote root growth even under N-limited conditions. Our results are consistent with those observed in serval studies which have indicated that PGPR inoculations effectively increased the root length and surface area (Bashan et al. 2004; Canbolat et al. 2006) indicating that this was a result of the synthesis from phytohormones and other secretions (Vacheron et al. 2013). The corn hybrid used in this experiment has a high root strength (root strength scale with 8 of 10) which means it has capacity to grow a strong root system. Therefore the corn hybrid may have masked some of the 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

(Sattelmacer et al. 1990; Kramer and Boyer 1995) and may exhibit higher water retention (El Zemrany et al. 2007). Several studies have reported that root structure and morphology could be influenced by soil microorganisms such as rhizobacteria (Gamalero et al. 2004; Lemanceau et al. 2005; El Zemrany et al. 2007; Calvo et al. 2017). El Zemrany et al. (2007) 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. (2017) reported that *Bacillus* spp. mixture 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.

5.4.3 Biomass Accumulation and N Uptake

Significant differences were observed among N levels for biomass of the root, stem, and leaf. Plant aboveground biomass tended to increase with increasing N rate at the V6 and VT stages, and no significant differences were observed at the V4 stage during both experimental times (Table 6, Figs. 1 and 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 (Fig. 1a). At the V6 and VT stages, the relatively high N rates (N75 and N100) had the largest plant biomass regardless of PGPR application. The full N rate treatment increased stem and leaf biomass by 32.4 ($P = 0.0124$) and 39.9% ($P = 0.0002$), respectively, 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. Plants with 50 and 75% of the recommended N rate also showed significant increases in root, stem, and leaf ($P < 0.0001$) biomass compared to the unfertilized control at the VT stage. Moreover, application of 50 and 75% of the recommended N rate can produce same plant biomass to that of the full rate treatment. Although there were no significant effects of 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 various N levels during the growing period (Table 6, Figs. 1 and 2). Both PGPR mixtures caused 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. Nitrogen fertilizer and PGPR interactions were observed for plant biomass accumulation at the V6 stage in HP1 (Fig. 1). PGPR mixture 1 with no N fertilizer (N0P1) had the greatest root, stem, and leaf biomass. Although there were no significant differences observed compared to the non-inoculation control, increases of 34.8 ($P = 0.0339$), 63.0 ($P = 0.0202$), and 41.3% ($P = 0.0283$) were noticed when compared to PGPR mixture 2, respectively. PGPR mixture 2 with 50% of recommended N (N50P2) had the greatest stem and leaf biomass with increases of 34.4 ($P = 0.0461$) and 25.6% ($P = 0.0495$) compared to the N50P0 treatment, respectively. However, at 75% of the recommended N rate, inoculation of PGPR strains had no benefit on aboveground biomass accumulation. This treatment even showed a lower stem and leaf biomass than the no-PGPR control. These results indicated that PGPR inoculation caused an increase in plant biomass that was slightly greater than the non-PGPR treatment at the various 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 application rate regardless of whether or not the PGPR inoculants were added at the VT stage for both experimental times (Table 6 and Fig. 3). Plants receiving 75% of the recommended N rate and the full recommended N rate significantly increased root, stem, and leaf N concentrations compared to 25% of recommended N rate and the unfertilized control, while the half N rate also significantly increased plant tissue N concentrations compared to the unfertilized control. These results indicate that under normal glasshouse conditions, 50 or 75% of the recommended N rate could satisfy the plant N requirement during the vegetative growth stages, especially at 75% of the recommended N rate application. This outcome may mask the positive effects of inoculated PGPR strains (Lin et al. 2017). No significant differences were observed for the response of corn N concentrations to PGPR inoculation (Fig 3). Also, there was no N application rate and PGPR interaction observed for plant tissue N concentrations. However, PGPR applications resulted in equivalent or greater N concentrations compared to non-PGPR treatments under low N level conditions, while a slightly lower N concentration was observed when PGPR inoculations were combined with relatively high N rates. This may be due to the dilution effect from greater plant tissue biomass. The results of leaf N concentration are consistent with the results of SPAD readings (Tables 2 and 3) due to the high positive correlation between these two parameters (Subedi and Ma, 2009; Zhu et al. 2011; Calvo et al. 2017). These results indicate the capacity of PGPR for improving 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, and enhancing root growth (root length and surface area) for nutrient absorption (Mantelin and Touraine 2004; Idris et al. 2007; Calvo 2013).

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 inoculations had a tendency to improve plant tissue N concentrations. Our results for plant biomass and N concentration are consistent with previous studies that have shown the positive effects of PGPR inoculation on plant dry weight and N uptake of corn (Biari et al. 2008; Calvo 2013; Huang et al. 2015; Marini et al. 2015; Kuan et al. 2016). Biari et al. (2008) indicated that inoculation of PGPR strains could not only increase corn growth parameters, such as plant height and shoot dry weight, but also enhanced grain dry weight and seed quality (100-seed weight and nutrients content). Therefore, the PGPR treatments in our experiment that enhanced plant growth parameters and biomass accumulation could increase 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 (Biari et al. 2008; Calvo et al. 2017). Consistently, a stronger root system, greater SPAD reading and dry below- and above-ground weight were observed with PGPR application in our experiment.

5.5 Conclusions

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 those of the full N fertilization rate, under glasshouse 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 consider as tools that will complement nutrient efficiency practices by increasing plant nutrient uptake efficiency, and therefore reduce N losses. Further studies are needed in order to know 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. Also, optimal field management practices for simulating the efficacy of PGPR under field conditions should be investigated.

5.6 References

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Table 1. Bacteria species and strains present in the PGPR mixtures used in this study.

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	*Paenibacillluspeoriae*

†PGPR strains were selected by screening testing (IAA production, ammonia production, phosphate solubilization, siderophore production, germination test, and seedling test).

Table 2. PGPR effects on corn plant height, stem diameter, leaf area, and SPAD reading as influenced by N rate during the V4, V6, and VT growth stages at the HP location from March to May (HP1).

Trt†	Plant height (cm)			Stem diameter (mm)			Leaf area (cm^2)			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 b‡	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 a	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 a	27.8 ± 0.84
N75P0	39.0 ± 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 a	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 b	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 b	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	0.0086	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 $P < 0.05$.

Table 3. PGPR effects on corn plant height, stem diameter, leaf area, and SPAD reading as influenced by N rate during the V4, V6, and VT growth stages at the HP location from April to June (HP2).

Trt†	Plant height (cm)			Stem diameter (mm)			Leaf area (cm^2)			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 ± 0.65	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	444.1 ± 52.4	1001 ± 63.0	2465 ± 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	13.58 ± 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)											
N	0.3594	0.8977	0.5929	0.7519	0.1994	0.0334	0.8151	0.5926	0.2731	0.1094	0.5412	<0.0001
PGPR	0.202	0.714	0.24	0.8773	0.0987	0.8182	0.909	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.

Table 4. PGPR effects on corn root morphology as influenced by N rate during the V4, V6, and VT growth stages at the HP location during March to May (HP1).

Trt†	Total length (cm)			Average diameter (mm)			Surface area (cm^2)			Total volume (cm^3)		
	V4	V6	VT	V4	V6	VT	V4	V6	VT	V4	V6	VT
N0P0	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
N0P1	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
N0P2	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
N25P0	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
N25P1	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
N25P2	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
N50P0	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
N50P1	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
N50P2	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
N75P0	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
N75P1	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
N75P2	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
N100P0	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
N100P1	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
N100P2	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	0.2864	0.0604	0.0412	0.4312	<0.0001	0.1202	0.1664	<0.0001	0.1967	0.2535	<0.0001
PGPR	0.416	0.9815	0.7626	0.0439	0.1126	0.6854	0.7262	0.3111	0.8359	0.9316	0.1713	0.6623
N*PGPR	0.6569	0.7563	0.5841	0.3377	0.1865	0.8089	0.5252	0.5395	0.4621	0.3702	0.3284	0.7938

†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 $P < 0.05$.

Table 5. PGPR effects on corn root morphology as influenced by N rate during the V4, V6, and VT growth stages at the HP location during April to June (HP2).

Trt†	Total length (cm)			Average diameter (mm)			Surface area (cm^2)			Total volume (cm^3)		
	V4	V6	VT	V4	V6	VT	V4	V6	VT	V4	V6	VT
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	0.2339	0.0745	0.0291	0.6678	0.2817	0.1337	0.8729	0.5132	0.4944	0.9626	0.5947	0.6813
PGPR	0.3883	0.0706	0.1087	0.6138	0.8035	0.7713	0.4553	0.1557	0.0832	0.6506	0.3565	0.3513
N*PGPR	0.4841	0.015	0.0168	0.7252	0.0951	0.819	0.7798	0.1833	0.3897	0.8489	0.1914	0.8754

†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 $P < 0.05$.

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 at the HP location during March to May (HP1) and April to June (HP2).

Source	P > F (0.05)					
	HP1			HP2		
	Root	Stem	Leaf	Root	Stem	Leaf
	Biomass at the V4 stage					
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
	Biomass at the V6 stage					
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
	Biomass at the VT stage					
N	<0.0001	<0.0001	<0.0001	0.591	0.0050	0.0795
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 at the VT stage					
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

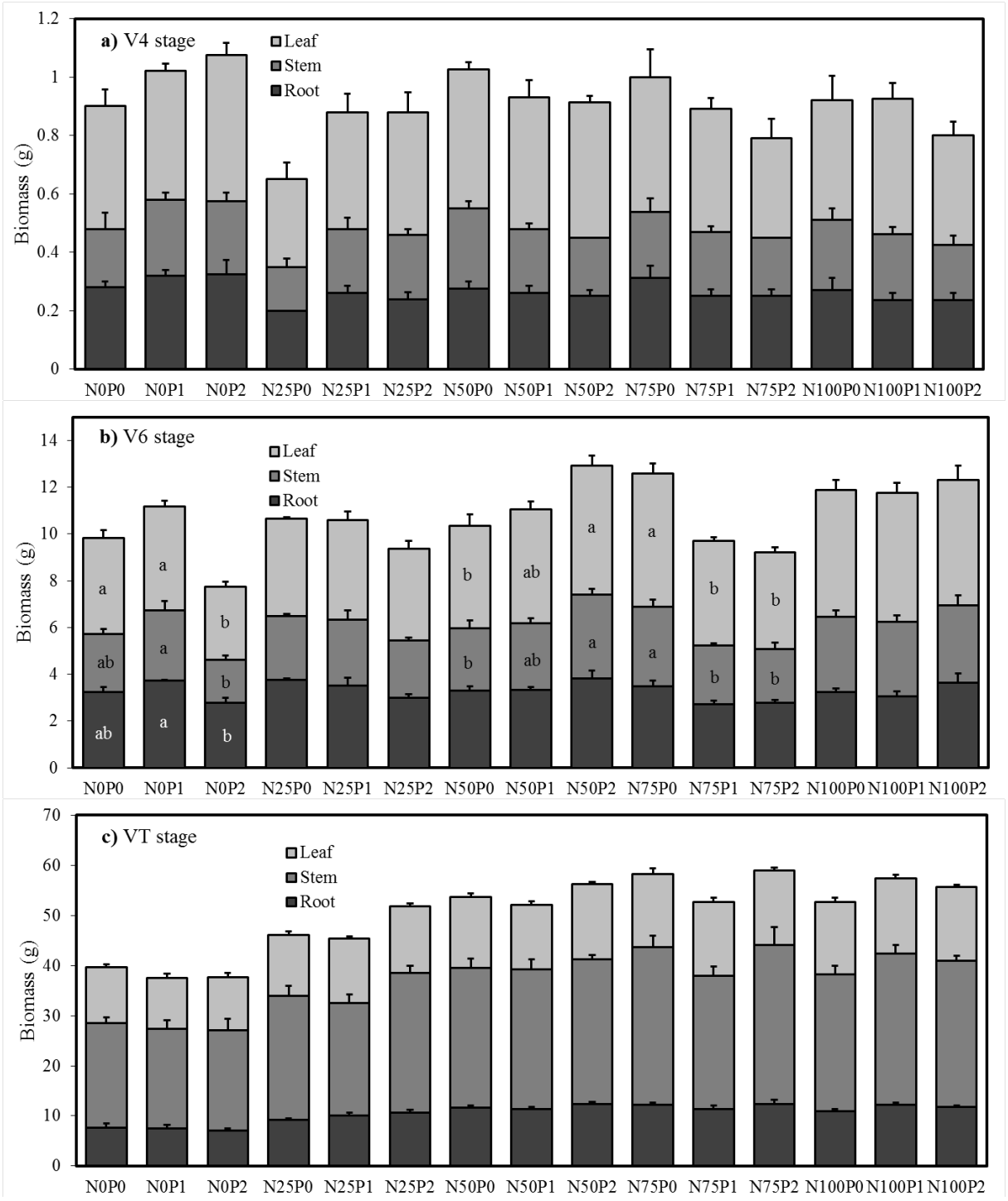

Fig. 1. Corn biomass (dry matter basis) for PGPR inoculation as influenced by N rate during a) V4, b) V6, and c) VT growth stages at the HP location from March to May (HP1). Data represent means and standard errors of replicates. Within each experimental time, bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$.

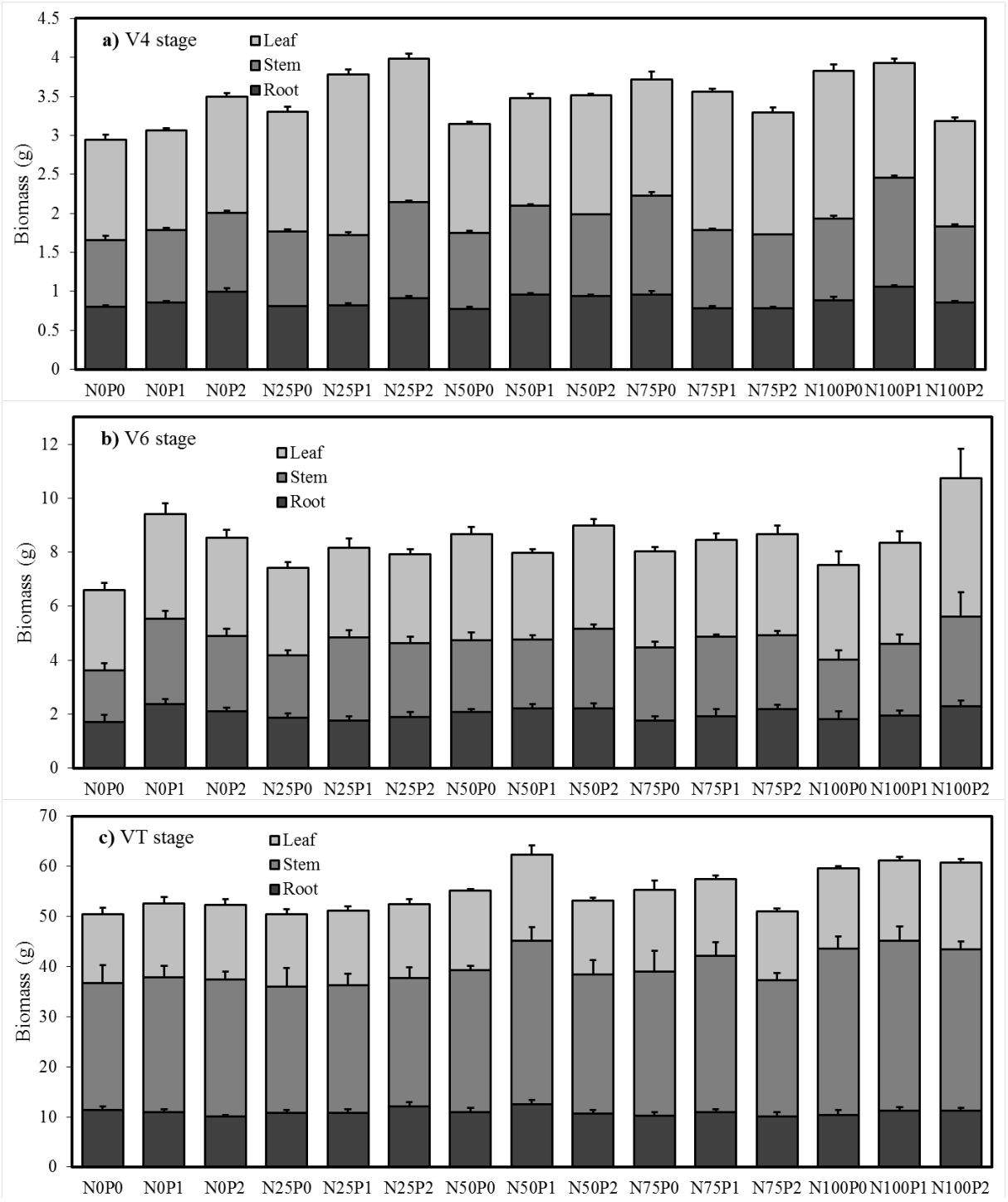

Fig. 2. Corn biomass (dry matter basis) for PGPR inoculation as influenced by N rate during a) V4, b) V6, and c) VT growth stages at the HP location from April to June (HP2). Data represent means and standard errors of replicates. Within each experimental time, bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$.

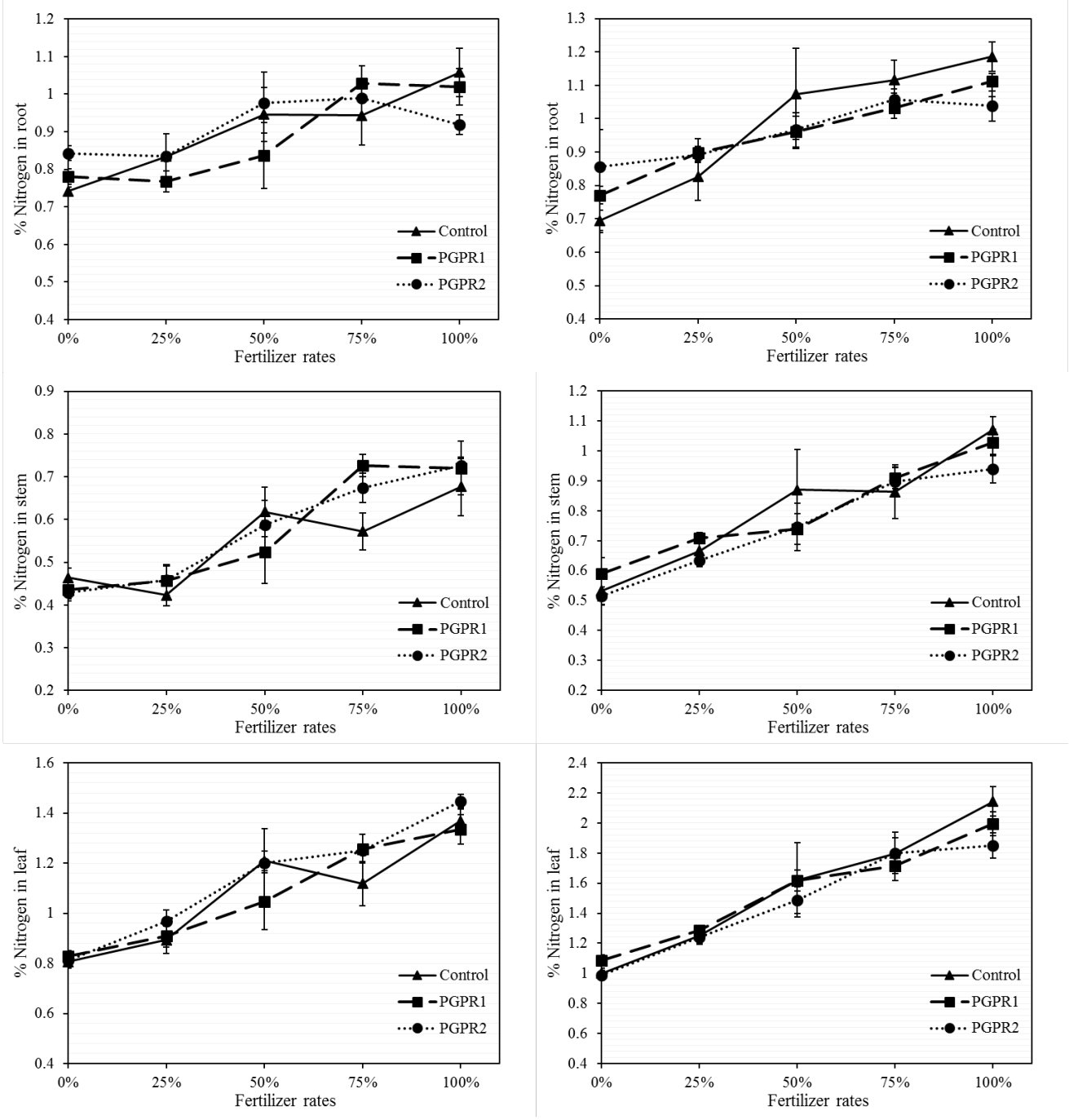

Fig. 3. Nitrogen concentration (%) in root, stem, and leaf for PGPR inoculation as influenced by N rate at the VT stage in the HP location from March to May (left) and from April to June (right). Fertilizer rates are percentages of the 100% rate (135 kg N ha^{-1}) recommended by Alabama Cooperative Extension System for corn on a Coastal Plain soil. Data represent means and standard errors of replicates. Within each experimental time, bar segments denoted by the same letter or with no letter assignment are not significantly different at $P < 0.05$.

6. Effect of PGPR on Corn Growth under Drought Stress

6.1 Abstract

Water availability is a major constraint affecting the growth and yield of agricultural crops worldwide. Some studies have shown that some free-living bacteria found in the plant rhizosphere can improve the tolerance of plants during water stress under drought conditions. Here we report results from a glasshouse study that evaluated the effects of two mixtures of plant growth-promoting rhizobacteria (PGPR) on root establishment and biomass production of maize (*Zea mays* L.) during the early growth stages using two fertilizer sources under drought conditions. Treatments included three irrigation levels (watering every 3, 6, and 12 days), two fertilizer materials (poultry litter and urea) applied at 45 kg total N ha^{-1}, and two PGPR strain mixtures and a non-inoculated control. Irrigation significantly affected plant growth and biomass accumulation of maize at V6 to VT stages. Compared to poultry litter, urea application increased plant height, leaf greenness, leaf area, and plant biomass. PGPR significantly improved plant height, stem diameter, leaf greenness, and root morphology under drought stress conditions. Therefore, PGPR inoculation could stimulate plant development likely through the production of plant growth regulators by bacteria at the rhizosphere, thereby enhancing root development, which results in better absorption of water and nutrients from the soil. Future research is needed to investigate the efficacy of PGPR on crop growth under nutrient and water limited conditions often experienced in agricultural fields.

6.2 Introduction

Water stress is one of the main constraints limiting sustainable crop production worldwide (Vinocur and Altman 2005; Naveed et al. 2014). For example, 32 counties in Alabama were designated as primary natural disaster areas and 15 additional counties were classified as

contiguous because of an ongoing drought in 2016 (Moseley, 2016). In 2011, drought reduced crop yields and affected livestock, costing Texas farmers and ranchers more than $5 billion, a 28% loss compared to average revenues of the previous four years (Fannin 2011). Drought is expected to cause serious plant growth problems for crops on more than 50% of the earth's arable lands by 2050 (Ashraf 1994; Vinocur and Altman 2005; Kasim et al. 2013). In some regions rainfall events have begun to decrease and extreme temperature increases are becoming more prevalent potentially due to global warming. As a result, severe drought problems have been observed in cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybean (*Glycine max* L.) in many crop-producing area around the world (IPCC 2007; EEA 2011). Thus, considerable effort is being made within the scientific community to develop management strategies than will be able to overcome this constraint.

In the past decades, extensive research has been carried out to reduce the effect of drought stress on plant growth and production, such as development of drought-tolerant varieties, evaluating the influence of shifting crop planting dates, and changes in resource management practices (Venkateswarlu and Shanker 2009); however, most of these techniques are cost-sensitive. An alternative strategy to mitigate the harmful effects of drought stress on crops could be the use microorganisms, including arbuscular mycorrhizal fungus (AM fungi); also soil bacteria have been suggested (Marulanda et al. 2009; Forchetti et al. 2010).

Numerous studies have been conducted to investigate the role of plant growth promoting rhizobacteria (PGPR) in the management of biotic and abiotic stresses (Sandhya et al. 2010; Karlidag et al. 2011; Yildirimet al. 2011; Ngumbi and Kloepper 2016). The possible mechanism of plant drought tolerance induced by rhizobacteria include: (1) production of phytohormones like abscisic acid (ABA), gibberellic acid, cytokinins, and indole-3-acetic acid (IAA); (2) ACC

deaminase to reduce the level of ethylene in the roots; (3) induced systemic tolerance by bacterial compounds; (4) bacterial exopolysaccharides (Yang et al., 2009; Dimkpa et al., 2009; Timmusk and Nevo, 2011; Kim et al., 2013; Timmusk et al., 2014). Several studies have reported that PGPR could ameliorate plant growth under drought stress conditions and protect plants from the deleterious effects of water deficit (Timmusk and Wagner 1999; Wang et al. 2012; Lim and Kim 2013; Grover et al. 2014; Cohen et al. 2015). Lim and Kim (2013) indicated that *Bacillus licheniformis* K11 could alleviate drought stress in pepper (*Capsium annuum* L.) by producing auxin and ACC deaminase. Inoculation of *Bacillus* spp. in sorghum (*Sorghum bicolor*) under drought stress resulted in better soil moisture, increased shoot length, and root dry biomass, thereby improving sorghum seedling growth (Grover et al. 2014). Kasim et al. (2013) inoculated *Bacillus* and *Azospirillum* strains on wheat (*Triticum aestivum*) under progressive drought conditions by withholding water for 4, 5, or 7 days and found that PGPR increased seedling survival, improved fresh and dry biomass weights, and plant tissue water content under drought. Similar results were also observed in corn seedlings when applying different PGPR strains (Sandhya et al. 2010; Vardharajula et al. 2011; Yasmin et al. 2013; Naseem and Bano 2014). These studies indicate that PGPR improved physiological and biochemical parameters of corn seedlings such as relative water content, increased levels of proline, sugars, and free amino acids, and decreased electrolyte leakage and antioxidant enzyme activity, thereby improving plant growth and alleviating drought stress. However, most previous studies investigating the effects of PGPR on plant growth under drought stress were conducted using sterilized soil rather than a real field soil. Moreover, drought treatments in these studies were only conducted for several days at the seedling stages.

The efficacy of PGPR as inoculants on crop growth is influenced by many biotic and abiotic factors (de Souza et al. 2015). Soil health is one of the important factors that affects the inoculation efficacy due to several soil characteristics, including soil texture, soil moisture, soil nutrient pool, soil microbial population and diversity, and soil disturbance caused by field management practices. Poultry manure use as a nutrient source has been suggested to improve soil quality and potentially increase crop productivity (Kingery et al. 1994; Watts et al. 2010; Hirzel et al. 2007; Tewolde et al. 2009; Watts and Torbert 2011; Lin et al. 2017a). For example, continuous application of litter or manure has been shown to increase soil organic matter, and levels of C, N, P, K, Ca and Mg (Wood et al. 1996; Ginting et al. 2003; Mitchell and Tu 2004; Watts et al. 2010; Lin et al. 2017b), thus creating a reservoir of soil nutrients for several years after application. As a result, this resulting reservoir of soil nutrients becomes a food source for microbes. Not only can poultry manure influence microbial processes in soil, microbial application to soil can also stimulate the decomposition of organic wastes and residues, thereby releasing available nutrients for plants (Lu et al. 2012; Fereidooni et al. 2013). Billah and Bano (2015) reported that wheat seeds inoculated with PGPR and subsequently treated with rock phosphate-enriched poultry litter compost increased plant height, grain yield, P uptake, and seed P content over the uninoculated untreated control. Lin et al. (2017c) observed that applying poultry litter with *Bacillus* spp. increased plant dry biomass production and improved root morphological parameters of corn when compared to chemical fertilizer with inoculants at the same N rate (Lin et al. 2017c).

In addition, several studies have reported that poultry manure applications could minimize the negative impact of drought stress on crop production and improve water use efficiency (Farhad et al. 2013; Afshar et al. 2014; Mannan et al. 2016). However, little information was

found in evaluation of poultry manure and PGPR application on plant growth under drought condition. Therefore, the objectives of this study were to: (1) evaluate the impact of PGPR mixtures, subsequent with poultry litter or inorganic N application, on corn root growth and biomass under drought stress; (2) investigate the effects of the interaction of irrigation, fertility source, and PGPR inoculant on vegetative growth of corn.

6.3 Materials and Methods

A container study was conducted in a glasshouse at the USDA-ARS National Soil Dynamics Laboratory, Auburn, AL from July to September and repeated in the same glasshouse from August to October in 2017. The soil used for this study was a Kalmia sandy loam (fine-loamy over sandy, siliceous, semiactive, thermic Typic Hapludults) collected from the Alabama Agricultural Experiment Station's E. V. Smith Research Center-Plant Breeding Unit in Elmore County, near Tallassee, AL. Surface soil (0-15 cm depth) was collected in early-summer of 2017 from an area that had been previously under row crop production. The PL used for this study was collected from a local broiler producer using standard production practices and consisted of manure and a bedding material mixture of wood shavings or sawdust, or both. Soil and PL used in this study were submitted to the Auburn University Soil Testing Laboratory and analyzed for nutrient concentrations (Table 2) according to procedures described by Hue and Evans (1986). Briefly, soil pH was determined on a 1:1 soil:water suspension using a glass electrode meter. Total C and N were determined by dry combustion using a LECO Truspec (LECO Corp., St. Joseph, MI, USA). Concentrations of P, K, Ca, Mg, Na, Cu, Fe, Mn, and Zn were determined using the dry ash procedure for PL (Donohue 1983) and with a Mehlich-1 extracting solution for soil (Olsen and Sommers 1982); both were measured using an ICAP 9000 (Thermo Jarrell Ash,

Franklin, MA, USA). The temperature and relative humidity within the glasshouse are shown in Table 1.

The experimental design was a randomized complete block design with five replications for each treatment. Treatments consisted of two fertilizer sources, three irrigation levels, and three PGPR inoculants. The two fertility sources consisted of PL (3.9% N), and urea (46% N) as the inorganic N fertilizer (IF). Each fertility source was applied on the soil surface at a rate of 45 kg total N ha^{-1} and then incorporated into the soil to a depth of 5 cm. For the IF treatments, 45 kg P ha^{-1} and 45 kg K ha^{-1} were also applied based on the initial soil nutrient levels as suggested by Auburn University's Soil Testing Laboratory according to Alabama Cooperative Extension Systems recommendations (Mitchell and Huluka, 2012). Irrigation levels consisted of sufficient, limited, and deficit irrigation with watering every 3 (I3), 6 (I6), and 12 (I12) days (d), respectively. The irrigation regimens started at 21 days after planting (DAP). PGPR treatments consisted of two PGPR strain mixtures (P1 and P2; Table 3) and one control without PGPR. Strains used for this study were obtained from Auburn University's PGPR culture collection housed in the Department of Entomology and Plant Pathology (Auburn, AL, USA). These strains have been shown to have positive effects on plant growth when evaluated in previous screenings. The bacterial mixtures were prepared by mixing each spore suspension of each strain in equal concentrations to result in the bacterial mix with a final concentration of 1 x 10^6 spores ml^{-1}.

The experimental units consisted of plastic containers (8 L Gro Pro square pots, Sunlight Supply, Inc., Vancouver, WA, USA) that were 24 cm tall, measuring 23 x 23 cm at the top, and tapered to 18 x 18 cm at the base. The containers were filled with 12.5 kg of soil and watered to saturation based on five extra containers designated for this purpose. 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 to a depth of 5 cm into the moist soil, and 1 ml of suspension of the respective bacterial mixture was applied on top of each seed at planting. After germination, plants were thinned to one plant per container and watered every three days to saturation until 21 DAP, at which time the irrigation regimens began. Suspension of the respective PGPR mixture was applied to soil as a 5 ml aqueous solution around the seedlings 10 DAP. Beginning two weeks after seeding, containers were randomly rotated weekly to minimize micro-environmental variation among treatments. Temperature of the glasshouse was set to 24° C during the day and 17° C at night. Given that the temperature tended to moderately fluctuate, the average temperature and relative humidity observed within the glasshouse during the experiments are shown in Table 1.

During the study, plant height, stem diameter, number of leaves, and leaf greenness (SPAD readings) were measured on 33, 45, and 57 DAP for each treatment within the irrigation regimens. The corn plants were harvested after three irrigation cycles (57 DAP). Leaf area, root morphological features, and dry shoot and root weights were measured at harvest. Plant height was determined by measuring from the plant's base to the top of the newest fully developed leaf. Stem diameter was determined using high-precision digital calipers. Afterwards, plants were cut at the soil surface with handheld pruning shears. For each plant, all leaves were cut from the stem and placed on an area meter one-by-one (avoiding overlap) to determine leaf area. Leaf area was determined from the harvested plants using an area meter (LI-3100C Area Meter, LI-COR Biosciences, Lincoln, NE, USA). To determine dry weight, the above- or below-ground plant biomass was then placed into paper bags and dried in a forced-air drying oven at 55 ºC until the weight became constant. 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 10000 professional scanner (Seiko Epson Corp., Shinjuku, Tokyo, Japan). Each individual root system was evenly spread apart in a water bath on a transparent tray (30 x 40 cm width) and imaged at a resolution of 157.5 dots per cm as described by Bauhus and Messier (1999) and Costa et al. (2000). The following root characteristics were determined: total root length (cm), root surface area (cm^2), root volume (cm^3), and average root diameter (mm).

Statistical analyses for each response variable in this experiment were performed using analysis of variance (ANOVA) with the PROC GLM procedure of SAS 9.4 (SAS Institute Inc. 2013) to determine treatment effects. The Tukey's honestly significant difference test was used to identify significant differences among treatments. A significance level of $\alpha = 0.05$ was established *a priori*, and differences between 0.05 and 0.10 were considered significant trends. Significant differences ($P \leq 0.05$) were observed between the two experimental times, thus treatment means for each experiment were analyzed separately.

6.4 Results and Discussion

6.4.1 Plant Growth Promotion

Corn plant growth was significantly affected by irrigation, fertility, and PGPR inoculants during the vegetative growth stages (Tables 4-6). Significant differences were observed for the main effects of irrigation on plant height, stem diameter, and leaf greenness (SPAD reading) at each sampling and on leaf area at harvest (57 DAP). Both sufficient and limited irrigation (I3 and I6) produced taller plants than deficit irrigation (I12) after the first irrigation cycle (33 DAP), while after the second and third cycle (45 and 57 DAP), the three irrigation levels were significantly different from each other for plant height in both experiments. Both sufficient and

limited irrigation (I3 and I6) significantly increased plant stem diameter compared to deficit irrigation (I12) at 33 and 57 DAP in the first experiment (Exp. 1), while in the second experiment (Exp. 2) significant differences were observed between only the two irrigation levels (I3 and I12) at three of the samplings. In Exp. 1, only the I3 treatment had greater leaf greenness than the deficit irrigation, while in Exp. 2 both sufficient and limited irrigation increased leaf greenness compared to I12 at 33 and 45 DAP. Sufficient irrigation had significantly greener leaves than the limited and deficit irrigation level at 57 DAP. Similarly, sufficient irrigation significantly increased leaf area on average by 22.7 and 93.9% in both experiments when compared to the limited and deficit irrigation levels, respectively. The most improved plant growth parameters observed in this study were obtained from the sufficient irrigation level regardless of fertility or PGPR treatments. Previous research has also reported decreased plant growth of corn with reducing water application (Sandhya et al. 2010; Vardharajula et al. 2011; Naseem and Bano 2014). Fard et al. (2011) reported that drought stress negatively affected the plant growth parameters such as leaf area, plant height, and stem diameter by changing a series of morphological, physiological, and metabolic processes, leading to reduced yield.

The main effect of fertility was only significant on plant height and leaf greenness (Tables 4-6). Application of IF resulted in taller plants than applying PL at the same total N rate on 33 and 45 DAP, while no significant differences were observed at 57 DAP. The leaf greenness was significantly increased by IF application, which indicates that the plant tends to have more N in leaves with IF application. With the same total N rate, PL has less available N than IF at the time of application (Collins et al. 1999; Chadwick et al. 2000). The organic fraction of litter-N becomes available only after mineralization via soil microbial activities (Ma et al. 1999). Therefore, it is difficult to predict how much litter-N became available for plant uptake during

the duration of this study due to the complex mineralization processes and the influence of microbial strain additions.

Inoculation of PGPR mixture 1 (P1) significantly influenced corn plant height, on average, with an increase of 4.6 and 8.1% at 45 and 57 DAP in Exp. 2, respectively (Tables 5-6). In addition, both PGPR inoculants (P1 and P2) had greater stem diameters at 45 DAP in Exp. 2. On average, 6.6 and 5.2% greater stem diameter was observed with inoculation of P1 and P2, respectively, when compared to the uninoculated control (Table 5). Although no significant differences were observed for the other plant growth parameters or sampling times, PGPR inoculation showed a significant trend for increasing plant height at the early vegetative growth stage (33 DAP), and PGPR inoculation enhanced leaf area at the late vegetative stage (57 DAP). Several studies have reported similar results showing that inoculation of PGPR can increase plant height, leaf area, and plant stem diameter (Çakmakçi et al. 2006; Park et al. 2015; Sengupta et al. 2015; Lin et al. 2017c).

Interactions between irrigation, fertility, and PGPR inoculants were observed on some of the plant growth parameters at different sampling times (Tables 4-6). Plant height was significantly affected by the interaction of irrigation and fertility at 45 and 57 DAP in Exp. 1. Significant differences were observed between irrigation levels with IF application, while with PL application both I3 and I6 had taller plants than the I12 treatment. No significant differences were observed between I3 and I6. The difference in performance due to irrigation between the IF and PL indicated that PL application may reduce the limitation of water stress on vegetative corn growth. Plant stem diameter had a significant response to the interaction of irrigation and fertility at 45 and 57 DAP in Exp. 1. Both sufficient and limited irrigation increased stem diameter compared to deficit irrigation with either IF or PL fertilization. In addition, stem diameter was

significantly influenced by interactions of irrigation and PGPR inoculants at 45 and 57 DAP in Exp. 2. The significant stem diameter increases observed with PGPR application indicate that inoculation of microbial strains, especially PGPR mixture 1, could strengthen plant stems under drought stress in the late vegetative growth stages. Leaf greenness was significantly affected by the interaction of irrigation and fertility at 33 and 57 DAP. Both sufficient and limited irrigation increased SPAD readings compared to deficit irrigation under IF application, while with PL application the response of leaf greenness varied depending on irrigation level. The PL-I6 treatment had the greenest leaves when compared to the PL-I3 and PL-I12 treatments at 57 DAP in Exp. 2. The interaction of irrigation on PGPR inoculants was also observed on leaf greenness where PGPR mixture 1 increased SPAD readings with sufficient irrigation, while PGPR mixture 2 had greener leaves than the non-inoculation treatment under drought stress. The leaf area was significantly affected by interaction of irrigation and fertility in Exp. 1 and interaction of fertility and PGPR inoculants in Exp. 2. Inoculation of microbial strains increased leaf area compared to the uninoculated control under IF application, while there were no significant differences between PGPR treatments with PL application. In addition, little irrigation × fertility × PGPR inoculants interaction was observed between treatments.

The interactions of irrigation × PGPR and fertility × PGPR present in this study indicate the influence of fertilizer source and water stress due to the performance of PGPR. The results showed that application of poultry litter had no effect on the efficacy of PGPR on vegetative growth of corn, while similar effects were reported in previous studies with additional organic components, including compost, biochar, and manure (Saxena et al. 2013; Iqbal et al. 2016; Prasad et al. 2017). The different responses of PGPR were mainly due to the various C:N ratios and application rates. For instance, these previous studies showed that PL influenced the efficacy

of PGPR on plant growth due to changes in the C:N ratio. However, in our study the small amount of PL applied most likely did not have the capacity to change the soil properties and the C:N ratio; experimental units with PL were most likely similar to that of the soil (Table 2). Inoculation of PGPR strains improved plant growth of corn under limited and deficit irrigation conditions. Similar results have been reported by Sandhya et al. (2010), Naseem and Bano (2014), and Fan et al. (2015). They attributed improved corn growth from PGPR additions to an increase in the relative plant water content, production of proline, amino acids, and soluble sugars concentration in leaves under drought stress.

6.4.2 Root Morphology

Mean root morphologies for each treatment are shown in Tables 7 and 8. Irrigation, fertility, and PGPR inoculants influenced root morphological parameters. The main effects of irrigation and fertility were observed on root surface area, average root diameter, and total root volume. Sufficient and limited irrigation produced greater root surface area, average diameter, and total volume than the deficit irrigation treatment. Moreover, both I3 and I6 significantly increased total root length compared to the I12 treatment in Exp. 2. Root development, not only root characteristics (root length, diameter, surface area, fresh and dry biomass, deep rooting, and cortex thickness) but also behaviors (root turnover, metacutisation, hardening, and hydraulic conductivity), are strongly affected by drought stress (Franco 2011).

Microbial inoculation significantly influenced the main effect of total root length. PGPR mixture 2 significantly increased root length at both experimental times (Tables 7 and 8). The main effect of PGPR inoculants was also observed on root surface area and total root volume in Exp. 2. PGPR mixture 2 produced the greatest root surface area and total volume. Moreover, the selected PGPR strains significantly increased specific root length (SRL) in Exp. 1. Specific root

length is the length of roots per unit of root mass and is a frequently measured morphological parameter for fine roots. These results indicate that inoculation of the selected PGPR strains could improve and modify root growth and build strong root systems for nutrient and water adsorption. Previous research also reported similar results on stimulating root growth (Jacoud et al. 1999; German et al. 2000; El Zemrany et al. 2007; Calvo et al. 2017). A greenhouse pot study conducted by El Zemrany et al. (2007) showed that corn seeds inoculated with the PGPR *Azospirillum lipoferum* increased the number of root tips, amount of root branching, and cumulative root length, but did not change the average root diameter. Vacheron et al. (2013) reviewed the relationship between PGPR and root systems and reported that PGPR strains can modify root system architecture, especially enhancing lateral root branching and development of root hairs, by producing phytohormones and other signals.

The irrigation × fertility × PGPR inoculant interaction and combination of the effects were observed on some of the root morphological parameters (Tables 7 and 8). Total root length was significantly influence by irrigation × PGPR and fertility × PGPR interactions in Exp. 1. Inoculation of PGPR mixture 2 significantly increased root length by 16.7% on average when compared to the uninoculated control under limited irrigation conditions. PGPR mixture 2 had the longest root length with IF application, while no differences were observed between PGPR treatments with PL application. The interaction of irrigation and PGPR inoculants was observed on root surface area in Exp. 1. There was no significant difference between PGPR treatments under sufficient irrigation conditions, while inoculation of microbial strains increased root surface area under drought stress, especially for the deficit irrigation conditions, with increases of 25.2 and 13.9% by PGPR mixtures 1 and 2, respectively. Interaction of irrigation and fertility influenced average root diameter in Exp. 2. Both sufficient and limited irrigation had greater root

diameter than deficit irrigation with IF application, while there was no significant difference between irrigation treatments under PL application. Fertility and PGPR inoculant interaction was observed on total root volume in Exp. 2. Inoculation of PGPR mixture 2 significantly increased root volume compared to the uninoculated control under IF application, but no differences were observed under PL application. These results indicate that drought stress can stimulate the positive effect of PGPR on plant root growth, and PGPR strains inoculated with IF applications may have more positive effects on root morphological growth than that with PL application. Previous studies also presented similar results showing that PGPR can stimulate root growth such as root length and surface area, and with a larger surface area of active roots the inoculated plants might exhibit higher water retention to alleviate drought stress (German et al. 2000; El Zemrany et al. 2007; Sandhya et al. 2010; Naseem and Bano 2014).

6.4.3 Biomass Accumulation

Corn plant biomass was significantly affected by irrigation and fertility (Fig. 1). On average, applying IF significantly increased corn root dry biomass ($P < 0.0001$ for Exp. 1 and $P = 0.008$ for Exp. 2), stem dry biomass ($P < 0.0001$ for Exp. 1 and $P = 0.0048$ for Exp. 2), and leaf dry biomass ($P < 0.0001$ for Exp. 1 and $P = 0.0001$ for Exp. 2) compared to PL. Our results were different when compared to several other studies which reported that application of PL resulted in an equivalent or greater plant biomass or grain yield of corn (Mitchell and Tu 2005; Watts and Torbert 2011; Lin et al. 2017a). In our experiment, both IF and PL applied at the same total N rate with 25% of the recommended N rate, thus, the available N in PL may not have satisfied plant N requirement during the growth stages. The lower SPAD readings observed in PL treatments (Tables 4-6) also indicate the lower N uptake from PL-amended soil. Significant differences were observed on root, stem, and leaf dry biomass between irrigation levels ($P <$

0.0001) in both experimental times. Our findings were consistent with those of other studies which suggests that biomass or yield reduction by drought stress are due to reduced nutrient diffusion and mass flow of water-soluble nutrients such as nitrate, sulfate, calcium, magnesium, and silicon (Barber 1995; Selvakumar et al. 2012) and influenced biochemical activities (Caravaca et al. 2008; Vurukonda et al. 2016). Although no significant differences were observed by PGPR inoculants, plants with PGPR strains tended to have greater biomass accumulation, such as the root and stem dry biomass in Exp. 1. Several published research studies have reported increased dry root biomass with inoculation of specific PGPR strains, but no difference on shoot biomass between PGPR inoculants and uninoculated control (Pan et al. 1999; Egamberdiyeva 2007; Myresiotis et al. 2015). The efficacy of bacterial inoculants on the stimulation of plant growth may have been affected by the soil conditions. For example, a pot study was conducted using calcisol soil and loamy sand with different N rates on corn production and results showed that PGPR strains had a much better stimulatory effect on plant biomass accumulation in the nutrient-deficient calcisol soil (Egamberdiyeva, 2007).

Corn plant dry biomass was significantly affected by the interaction between irrigation and fertility in Exp. 1 (Fig.1). The greatest root biomass was observed in the limited irrigation treatment with IF application ($P = 0.0026$), while the greatest stem ($P < 0.0001$) and leaf ($P = 0.0032$) biomass were observed in the sufficient irrigation treatment with IF application. In addition, a significant difference was observed between sufficient and limited irrigation with IF application, while there was no significant difference between these two irrigation levels (I3 and I6) with PL application. Root ($P = 0.0139$) and stem ($P = 0.0326$) biomass accumulation were significantly affected by the interaction of irrigation × fertility × PGPR inoculants in Exp. 1.

However, no irrigation × fertility × PGPR inoculants interaction or any combination of the effects was observed between treatments in Exp. 2.

6.5 Conclusion

It is concluded that limited and deficit irrigation significantly affected plant growth and biomass accumulation of corn. Compared to poultry litter, inorganic N application increased plant height, leaf greenness, leaf area, and plant dry biomass when at a low N application rate. The selected PGPR mixtures significantly increased root morphological parameters and they improved some of the above- and below-ground plant growth parameters under drought stress conditions. Therefore, PGPR inoculation could stimulate plant development through the production of plant growth regulators by bacteria at the rhizosphere, which could enhance root development, resulting in better absorption of water and nutrients from the soil. More work is needed on these findings in field conditions to further investigate the efficacy of PGPR on crop growth under nutrient- and water-limiting conditions.

6.6 References

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Table 1. Temperature and relative humidity in the glasshouse during the experimental periods.

Duration	Average temperature (°C)				Average relative humidity (%)			
	Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	Day	Night	Day	Night	Day	Night	Day	Night
0 - 21 DAP	28.36	25.50	29.64	25.99	77.78	84.31	76.26	85.48
21 - 33 DAP	29.50	25.68	26.51	23.29	78.77	89.00	73.10	80.46
33 - 45 DAP	28.56	25.57	26.21	22.07	75.19	81.26	73.05	82.97
45 - 57 DAP	25.58	21.86	27.24	23.32	70.15	78.54	69.95	77.73

Table 2. Physical and chemical properties of initial soil and poultry litter on a dry weight basis.

Property	Initial soil	Poultry litter
Bulk density (g cm^{-3})	1.30	-
pH (1:1 soil:water)	6.2	-
Moisture content (g 100 g^{-1})	-	28.1
Total C (g kg^{-1})	4.75	333.8
Total N (g kg^{-1})	0.50	38.5
C:N ratio	9.5	8.67
P (g kg^{-1}) †	0.02	17.7
K (g kg^{-1})	0.07	28.5
Ca (g kg^{-1})	0.22	35.9
Mg (g kg^{-1})	0.05	7.50
Na (g kg^{-1})	-	13.8
Cu (mg kg^{-1})	0.20	177
Fe (mg kg^{-1})	12.0	2532
Mn (mg kg^{-1})	10.5	477
Zn (mg kg^{-1})	0.55	493

†P, K, Ca, Mg, Na, Cu, Fe, Mn, and Zn values represent Mehlich-1 extractable nutrient concentrations for soil and total nutrient concentrations for poultry litter.

Table 3. Bacteria species and strains present in the PGPR mixtures used in this study.

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	*Paenibacillluspeoriae*

†PGPR strains were selected by screening testing (IAA production, ammonia production, phosphate solubilization, siderophore production, germination test, and seedling test).

Table 4. The effects of N source, irrigation, and PGPR on plant height, stem diameter, and SPAD readings of corn at 33 DAP.

Treatments†	Plant Height (cm)		Stem Diameter (mm)		SPAD reading	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
IF-I3-P0	92.7 ± 5.37‡	78.8 ± 1.73	14.7 ± 0.56	14.7 ± 0.64	40.7 ± 2.03	42.5 ± 2.07
IF-I3-P1	79.8 ± 9.13	79.6 ± 4.46	14.5 ± 0.83	14.6 ± 0.44	43.3 ± 1.77	42.2 ± 0.90
IF-I3-P2	81.0 ± 2.40	82.8 ± 2.48	15.8 ± 0.77	14.4 ± 0.57	42.3 ± 0.47	41.8 ± 0.97
IF-I6-P0	85.9 ± 4.72	66.3 ± 0.89	14.9 ± 0.53	13.1 ± 0.29	39.8 ± 0.60	44.8 ± 1.25
IF-I6-P1	86.3 ± 4.02	73.8 ± 2.48	14.9 ± 0.50	13.5 ± 0.51	41.4 ± 0.82	42.1 ± 1.24
IF-I6-P2	74.4 ± 2.49	79.5 ± 4.14	15.6 ± 0.25	13.7 ± 0.38	41.8 ± 0.22	44.5 ± 0.88
IF-I12-P0	72.8 ± 2.62	67.2 ± 3.46	13.0 ± 0.36	13.0 ± 0.30	39.3 ± 1.42	37.7 ± 0.79
IF-I12-P1	73.9 ± 2.50	67.7 ± 4.53	13.0 ± 0.16	12.3 ± 0.44	41.1 ± 0.87	39.3 ± 0.73
IF-I12-P2	72.9 ± 4.67	68.4 ± 4.03	12.5 ± 0.40	12.6 ± 0.59	37.2 ± 2.28	38.5 ± 1.27
PL-I3-P0	79.7 ± 5.09	78.1 ± 3.21	15.0 ± 0.23	15.0 ± 0.54	36.4 ± 2.06	44.5 ± 1.22
PL-I3-P1	81.6 ± 6.15	76.8 ± 4.08	15.2 ± 0.55	14.9 ± 0.30	34.4 ± 2.41	43.8 ± 1.32
PL-I3-P2	75.9 ± 3.23	78.8 ± 1.65	14.8 ± 0.44	14.7 ± 0.41	32.3 ± 2.00	39.1 ± 1.25
PL-I6-P0	79.6 ± 3.82	75.0 ± 3.46	14.0 ± 0.46	12.9 ± 0.28	34.0 ± 1.94	40.7 ± 1.11
PL-I6-P1	74.4 ± 4.85	78.5 ± 4.61	15.5 ± 0.31	13.4 ± 0.45	37.0 ± 1.80	40.4 ± 0.22
PL-I6-P2	80.1 ± 2.34	76.8 ± 3.97	15.2 ± 0.55	12.5 ± 0.35	36.3 ± 2.78	39.9 ± 1.27
PL-I12-P0	66.5 ± 3.28	65.5 ± 4.12	13.7 ± 0.69	12.1 ± 0.63	33.3 ± 0.38	37.2 ± 1.26
PL-I12-P1	57.1 ± 1.93	62.9 ± 3.58	13.9 ± 0.47	11.9 ± 0.96	32.7 ± 1.70	37.2 ± 0.67
PL-I12-P2	64.4 ± 2.87	71.7 ± 1.52	13.9 ± 0.67	11.4 ± 0.34	33.1 ± 1.38	40.0 ± 1.61
	P > F (0.05)					
Fertility (F)	0.0011	0.9971	0.2728	0.1402	< 0.0001	0.037
Irrigation (I)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0406	< 0.0001
PGPR (P)	0.098	0.0922	0.3441	0.637	0.449	0.6655
F * I	0.3821	0.3116	0.1003	0.1658	0.4361	0.0157
F * P	0.3305	0.6852	0.403	0.5844	0.6342	0.647
I * P	0.7308	0.6689	0.5612	0.7524	0.5819	0.0395
F * I * P	0.1398	0.5128	0.487	0.9281	0.399	0.1144

†IF- inorganic fertilizer; PL- poultry litter; I3, I6, I12- irrigate every 3, 6, and 12 days, respectively; P0- no PGPR control; P1 and P2- PGPR mixtures 1 and 2.

‡ Data represent means and standard errors of replicates.

Table 5. The effects of N source, irrigation, and PGPR on plant height, stem diameter, and SPAD readings of corn at 45 DAP.

Treatments†	Plant Height (cm)		Stem Diameter (mm)		SPAD reading	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
IF-I3-P0	144 ± 9.65‡	119 ± 6.28	14.8 ± 0.81	13.5 ± 0.38	33.3 ± 1.28	34.4 ± 1.65
IF-I3-P1	131 ± 12.7	130 ± 1.81	13.9 ± 0.30	15.0 ± 0.39	34.9 ± 2.54	34.9 ± 2.01
IF-I3-P2	144 ± 1.26	131 ± 2.27	16.1 ± 0.89	13.8 ± 0.50	36.4 ± 0.94	33.1 ± 3.12
IF-I6-P0	113 ± 9.22	96.5 ± 2.40	13.3 ± 0.51	11.3 ± 0.19	32.8 ± 2.94	32.0 ± 2.29
IF-I6-P1	105 ± 1.82	99.6 ± 1.94	14.5 ± 0.24	12.1 ± 0.26	34.6 ± 1.89	33.8 ± 1.36
IF-I6-P2	106 ± 3.81	98.6 ± 2.29	14.0 ± 0.34	12.1 ± 0.10	31.7 ± 1.24	33.0 ± 1.95
IF-I12-P0	94.4 ± 2.83	87.6 ± 3.06	10.9 ± 0.37	9.69 ± 0.12	25.9 ± 3.40	23.4 ± 1.89
IF-I12-P1	88.8 ± 4.12	84.0 ± 3.44	9.94 ± 0.31	9.86 ± 0.48	28.0 ± 2.55	23.0 ± 2.95
IF-I12-P2	85.1 ± 2.41	83.8 ± 1.55	10.9 ± 0.76	11.1 ± 0.28	28.6 ± 1.69	26.1 ± 0.40
PL-I3-P0	116 ± 11.4	120 ± 2.14	14.1 ± 0.39	13.3 ± 0.09	33.2 ± 2.53	31.5 ± 0.83
PL-I3-P1	111 ± 4.75	120 ± 1.22	14.8 ± 0.69	14.5 ± 0.29	29.3 ± 1.56	31.9 ± 1.90
PL-I3-P2	110 ± 2.87	118 ± 2.89	14.4 ± 0.24	13.5 ± 0.51	27.7 ± 0.84	33.1 ± 0.88
PL-I6-P0	97.0 ± 6.07	97.8 ± 2.22	13.3 ± 0.37	11.5 ± 0.12	27.8 ± 1.67	28.3 ± 4.26
PL-I6-P1	98.9 ± 7.58	103 ± 1.36	13.7 ± 0.41	12.2 ± 0.19	31.9 ± 2.11	29.7 ± 1.66
PL-I6-P2	104 ± 4.45	90.2 ± 1.88	14.6 ± 0.64	12.2 ± 0.19	32.7 ± 1.71	28.4 ± 1.96
PL-I12-P0	93.9 ± 5.24	76.8 ± 4.29	12.2 ± 0.26	9.88 ± 0.22	29.0 ± 2.42	26.3 ± 1.15
PL-I12-P1	78.2 ± 1.36	87.8 ± 4.17	11.5 ± 0.08	9.93 ± 0.37	25.6 ± 1.38	26.0 ± 1.47
PL-I12-P2	85.4 ± 5.59	78.0 ± 2.39	11.3 ± 0.65	9.96 ± 0.46	23.8 ± 2.56	25.0 ± 0.80
	P > F (0.05)					
Fertility (F)	< 0.0001	0.0026	0.4772	0.3089	0.0061	0.1351
Irrigation (I)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PGPR (P)	0.1164	0.0172	0.1849	0.0004	0.8921	0.8851
F * I	0.0032	0.2004	0.0298	0.3552	0.3464	0.0689
F * P	0.88	0.059	0.4195	0.4316	0.2792	0.9647
I * P	0.8269	0.3756	0.1375	0.0072	0.599	0.9587
F * I * P	0.482	0.0337	0.1071	0.5068	0.0869	0.7741

†IF- inorganic fertilizer; PL- poultry litter; I3, I6, I12- irrigate every 3, 6, and 12 days, respectively; P0- no PGPR control; P1 and P2- PGPR mixtures 1 and 2.

‡ Data represent means and standard errors of replicates.

Table 6. The effects of N source, irrigation, and PGPR on plant height, stem diameter, SPAD readings, and leaf area of corn at 57 DAP.

Treatments†	Plant Height (cm)		Stem Diameter (mm)		SPAD reading		Leaf Area (cm^2)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
IF-I3-P0	245 ± 13.5‡	205 ± 13.4	14.1 ± 0.66	13.3 ± 0.41	32.0 ± 2.16	23.3 ± 1.64	3009 ± 211	2377 ± 161
IF-I3-P1	222 ± 38.6	224 ± 3.76	13.9 ± 0.23	14.2 ± 0.50	28.4 ± 4.48	24.5 ± 1.79	2852 ± 295	2663 ± 49.6
IF-I3-P2	249 ± 13.3	223 ± 9.17	15.4 ± 0.61	12.9 ± 0.67	31.0 ± 4.28	25.7 ± 2.79	2915 ± 133	2559 ± 113
IF-I6-P0	163 ± 17.2	154 ± 7.63	14.2 ± 0.62	11.8 ± 0.34	28.1 ± 1.10	29.5 ± 1.08	2557 ± 132	1714 ± 68.5
IF-I6-P1	152 ± 7.95	169 ± 5.04	13.8 ± 0.51	12.0 ± 0.10	29.0 ± 0.70	30.6 ± 2.37	2517 ± 113	1877 ± 44.7
IF-I6-P2	156 ± 14.9	151 ± 2.40	13.3 ± 0.41	12.8 ± 0.16	25.4 ± 2.07	27.3 ± 2.23	2239 ± 81.6	1996 ± 105
IF-I12-P0	115 ± 17.6	77.6 ± 2.18	10.8 ± 0.64	6.86 ± 0.26	21.0 ± 3.09	18.7 ± 2.00	1609 ± 143	1118 ± 52.3
IF-I12-P1	84.4 ± 5.90	77.4 ± 3.31	10.0 ± 0.34	7.45 ± 0.45	17.7 ± 1.47	15.6 ± 2.45	1655 ± 134	1205 ± 50.4
IF-I12-P2	84.3 ± 2.93	78.8 ± 2.93	9.79 ± 0.28	8.18 ± 0.53	18.8 ± 1.55	17.2 ± 1.21	1479 ± 113	1137 ± 28.8
PL-I3-P0	180 ± 27.4	197 ± 5.55	13.6 ± 0.43	14.0 ± 0.40	25.2 ± 6.50	21.0 ± 1.85	2013 ± 178	2477 ± 70.9
PL-I3-P1	190 ± 16.8	215 ± 2.74	14.3 ± 0.81	13.6 ± 0.43	27.7 ± 3.31	14.6 ± 1.05	2141 ± 221	2457 ± 96.1
PL-I3-P2	180 ± 7.58	206 ± 8.83	13.3 ± 0.21	13.2 ± 0.25	19.1 ± 1.06	16.9 ± 3.09	1931 ± 78.7	2362 ± 42.2
PL-I6-P0	157 ± 10.7	152 ± 3.64	13.0 ± 0.37	12.4 ± 0.46	22.9 ± 1.64	25.7 ± 2.61	1944 ± 108	1959 ± 40.4
PL-I6-P1	154 ± 23.0	169 ± 4.27	13.5 ± 0.21	12.1 ± 0.18	18.7 ± 5.30	27.2 ± 2.78	2104 ± 143	1870 ± 45.8
PL-I6-P2	169 ± 19.6	154 ± 3.01	13.5 ± 0.54	12.3 ± 0.36	23.7 ± 1.52	26.9 ± 2.13	1918 ± 93.9	1746 ± 41.0
PL-I12-P0	132 ± 21.6	76.0 ± 1.22	11.5 ± 0.31	7.03 ± 0.08	26.7 ± 3.98	16.1 ± 1.78	1777 ± 112	1102 ± 34.4
PL-I12-P1	74.8 ± 5.55	77.4 ± 4.97	11.5 ± 0.49	7.58 ± 0.39	14.3 ± 3.96	19.0 ± 1.48	1183 ± 47.0	1173 ± 137
PL-I12-P2	94.5 ± 16.5	76.4 ± 3.64	10.9 ± 0.44	7.96 ± 0.29	24.2 ± 4.52	17.9 ± 0.97	1359 ± 102	919 ± 42.4
	P > F (0.05)							
Fertility (F)	0.0563	0.1571	0.8927	0.7018	0.0441	0.0032	< 0.0001	0.0945
Irrigation (I)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0033	< 0.0001	< 0.0001	< 0.0001
PGPR (P)	0.1632	0.0055	0.7765	0.291	0.2097	0.9119	0.0831	0.1122
F * I	0.004	0.1958	0.005	0.9756	0.0375	0.0087	0.0001	0.5273
F * P	0.9688	0.947	0.3244	0.3095	0.7694	0.9784	0.9311	0.0031
I * P	0.392	0.0873	0.485	0.0167	0.4069	0.6787	0.4662	0.6078
F * I * P	0.7045	0.9348	0.1923	0.6694	0.2054	0.1538	0.1332	0.527

†IF- inorganic fertilizer; PL- poultry litter; I3, I6, I12- irrigate every 3, 6, and 12 days, respectively; P0- no PGPR control; P1 and P2- PGPR mixtures 1 and 2.

‡ Data represent means and standard errors of replicates.

Table 7. The effects of N source, irrigation, and PGPR on corn root morphology and root-to-shoot ratio (RSR) at 57 DAP during June to September (Exp. 1).

Treatments†	Total length (cm)	Surface area (cm^2)	Average diameter (mm)	Total volume (cm^3)	SRL (m g^{-1})	RSR
IF-I3-P0	6185 ± 490‡	1672 ± 87.0	0.87 ± 0.04	36.1 ± 1.98	7.62 ± 1.24	0.19 ± 0.01
IF-I3-P1	6214 ± 241	1529 ± 154	0.79 ± 0.09	30.8 ± 6.77	11.0 ± 1.66	0.16 ± 0.01
IF-I3-P2	7592 ± 217	1656 ± 33.8	0.69 ± 0.01	28.7 ± 0.57	7.56 ± 0.43	0.20 ± 0.02
IF-I6-P0	5112 ± 254	1483 ± 39.2	0.78 ± 0.04	28.9 ± 2.27	7.70 ± 0.43	0.25 ± 0.01
IF-I6-P1	6678 ± 403	1615 ± 84.7	0.78 ± 0.06	31.7 ± 3.68	7.63 ± 0.27	0.26 ± 0.01
IF-I6-P2	7002 ± 290	1737 ± 33.9	0.76 ± 0.03	34.4 ± 1.28	9.08 ± 0.98	0.27 ± 0.03
IF-I12-P0	5883 ± 150	1172 ± 27.6	0.64 ± 0.03	18.2 ± 0.72	12.1 ± 0.65	0.24 ± 0.03
IF-I12-P1	6885 ± 479	1487 ± 65.6	0.70 ± 0.06	26.1 ± 2.85	18.1 ± 2.48	0.26 ± 0.03
IF-I12-P2	6473 ± 379	1405 ± 81.0	0.69 ± 0.01	24.3 ± 1.56	13.4 ± 0.56	0.28 ± 0.01
PL-I3-P0	6747 ± 325	1959 ± 138	0.93 ± 0.07	45.9 ± 5.96	11.0 ± 1.00	0.22 ± 0.03
PL-I3-P1	5441 ± 224	1715 ± 117	1.01 ± 0.07	43.6 ± 5.36	10.1 ± 1.03	0.19 ± 0.03
PL-I3-P2	5240 ± 305	1910 ± 99.6	1.18 ± 0.09	56.9 ± 6.92	10.1 ± 0.88	0.20 ± 0.01
PL-I6-P0	4742 ± 131	1646 ± 143	1.10 ± 0.09	46.6 ± 7.01	8.18 ± 0.48	0.23 ± 0.01
PL-I6-P1	5571 ± 145	1535 ± 202	0.87 ± 0.10	34.9 ± 7.93	9.18 ± 1.15	0.23 ± 0.02
PL-I6-P2	5666 ± 63.0	1580 ± 101	0.89 ± 0.06	35.6 ± 5.04	8.72 ± 0.85	0.22 ± 0.02
PL-I12-P0	5403 ± 71.9	1300 ± 54.7	0.77 ± 0.04	24.2 ± 1.57	12.4 ± 0.59	0.16 ± 0.01
PL-I12-P1	6118 ± 154	1607 ± 217	0.83 ± 0.10	34.6 ± 8.96	13.5 ± 0.43	0.33 ± 0.03
PL-I12-P2	5699 ± 407	1411 ± 31.2	0.80 ± 0.06	28.2 ± 2.30	12.7 ± 1.47	0.28 ± 0.03
	P > F (0.05)					
Fertility (F)	< 0.0001	0.0515	< 0.0001	< 0.0001	0.7059	0.6143
Irrigation (I)	0.2776	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PGPR (P)	0.0372	0.4424	0.8686	0.8698	0.0174	0.0659
F * I	0.2064	0.1014	0.2394	0.123	0.0277	0.1332
F * P	0.01	0.4025	0.4572	0.8409	0.0907	0.1697
I * P	0.0053	0.0413	0.2283	0.2713	0.1459	0.0045
F * I * P	0.0066	0.8501	0.0121	0.1127	0.1869	0.0778

†IF- inorganic fertilizer; PL- poultry litter; I3, I6, I12- irrigate every 3, 6, and 12 days, respectively; P0- no PGPR control; P1 and P2- PGPR mixtures 1 and 2.

‡ Data represent means and standard errors of replicates.

Table 8. The effects of N source, irrigation, and PGPR on corn root morphology and root-to-shoot ratio (RSR) at 57 DAP during August to October (Exp. 2).

Treatments†	Total length (cm)	Surface area (cm^2)	Average diameter (mm)	Total volume (cm^3)	SRL (m g^{-1})	RSR
IF-I3-P0	4753 ± 150‡	1264 ± 45.3	0.88 ± 0.06	26.0 ± 1.40	7.80 ± 1.09	0.18 ± 0.01
IF-I3-P1	5087 ± 362	1409 ± 24.3	1.07 ± 0.14	30.7 ± 2.83	6.97 ± 1.37	0.19 ± 0.02
IF-I3-P2	5216 ± 95.6	1659 ± 106	0.90 ± 0.05	45.8 ± 8.23	8.81 ± 1.24	0.18 ± 0.02
IF-I6-P0	5023 ± 112	1479 ± 61.7	0.82 ± 0.04	30.6 ± 2.56	10.7 ± 0.97	0.21 ± 0.01
IF-I6-P1	5367 ± 226	1482 ± 33.8	0.93 ± 0.06	34.3 ± 1.45	9.21 ± 0.80	0.25 ± 0.01
IF-I6-P2	5748 ± 212	1617 ± 32.5	0.91 ± 0.02	39.6 ± 1.53	9.88 ± 0.21	0.21 ± 0.01
IF-I12-P0	4536 ± 174	986 ± 18.9	0.70 ± 0.02	21.1 ± 1.23	14.9 ± 0.42	0.25 ± 0.01
IF-I12-P1	4256 ± 123	1049 ± 26.0	0.74 ± 0.02	19.3 ± 0.57	14.7 ± 0.26	0.23 ± 0.01
IF-I12-P2	5195 ± 298	1141 ± 43.0	0.74 ± 0.01	19.7 ± 1.36	14.1 ± 0.68	0.24 ± 0.01
PL-I3-P0	5453 ± 354	1319 ± 68.3	0.81 ± 0.03	26.0 ± 1.01	9.47 ± 1.00	0.19 ± 0.01
PL-I3-P1	5440 ± 398	1361 ± 110	0.80 ± 0.02	25.4 ± 2.68	9.95 ± 0.25	0.18 ± 0.01
PL-I3-P2	5478 ± 412	1415 ± 87.0	0.77 ± 0.02	27.2 ± 2.48	11.1 ± 0.40	0.16 ± 0.01
PL-I6-P0	5588 ± 119	1295 ± 37.2	0.80 ± 0.03	28.1 ± 2.29	10.7 ± 0.62	0.22 ± 0.01
PL-I6-P1	5380 ± 314	1369 ± 69.5	0.81 ± 0.03	25.9 ± 1.41	9.26 ± 0.40	0.22 ± 0.02
PL-I6-P2	5376 ± 97.2	1399 ± 61.5	0.80 ± 0.03	27.9 ± 1.98	11.0 ± 1.19	0.22 ± 0.02
PL-I12-P0	4841 ± 273	1052 ± 63.7	0.69 ± 0.04	18.4 ± 1.90	16.5 ± 1.82	0.24 ± 0.01
PL-I12-P1	4135 ± 203	1017 ± 84.6	0.71 ± 0.04	19.9 ± 1.62	15.61 ± 1.46	0.22 ± 0.01
PL-I12-P2	4871 ± 205	1099 ± 40.1	0.72 ± 0.04	17.0 ± 2.31	15.9 ± 1.09	0.25 ± 0.01
	P > F (0.05)					
Fertility (F)	0.2086	0.0072	0.0002	< 0.0001	0.0053	0.6034
Irrigation (I)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PGPR (P)	0.0402	0.0003	0.0886	0.0204	0.2985	0.6336
F * I	0.2332	0.0777	0.0423	0.1053	0.2829	0.794
F * P	0.0818	0.1335	0.121	0.0201	0.8609	0.2193
I * P	0.2086	0.5752	0.5801	0.0571	0.5542	0.2645
F * I * P	0.974	0.5662	0.6776	0.2102	0.9455	0.6545

†IF- inorganic fertilizer; PL- poultry litter; I3, I6, I12- irrigate every 3, 6, and 12 days, respectively; P0- no PGPR control; P1 and P2- PGPR mixtures 1 and 2.

‡ Data represent means and standard errors of replicates.

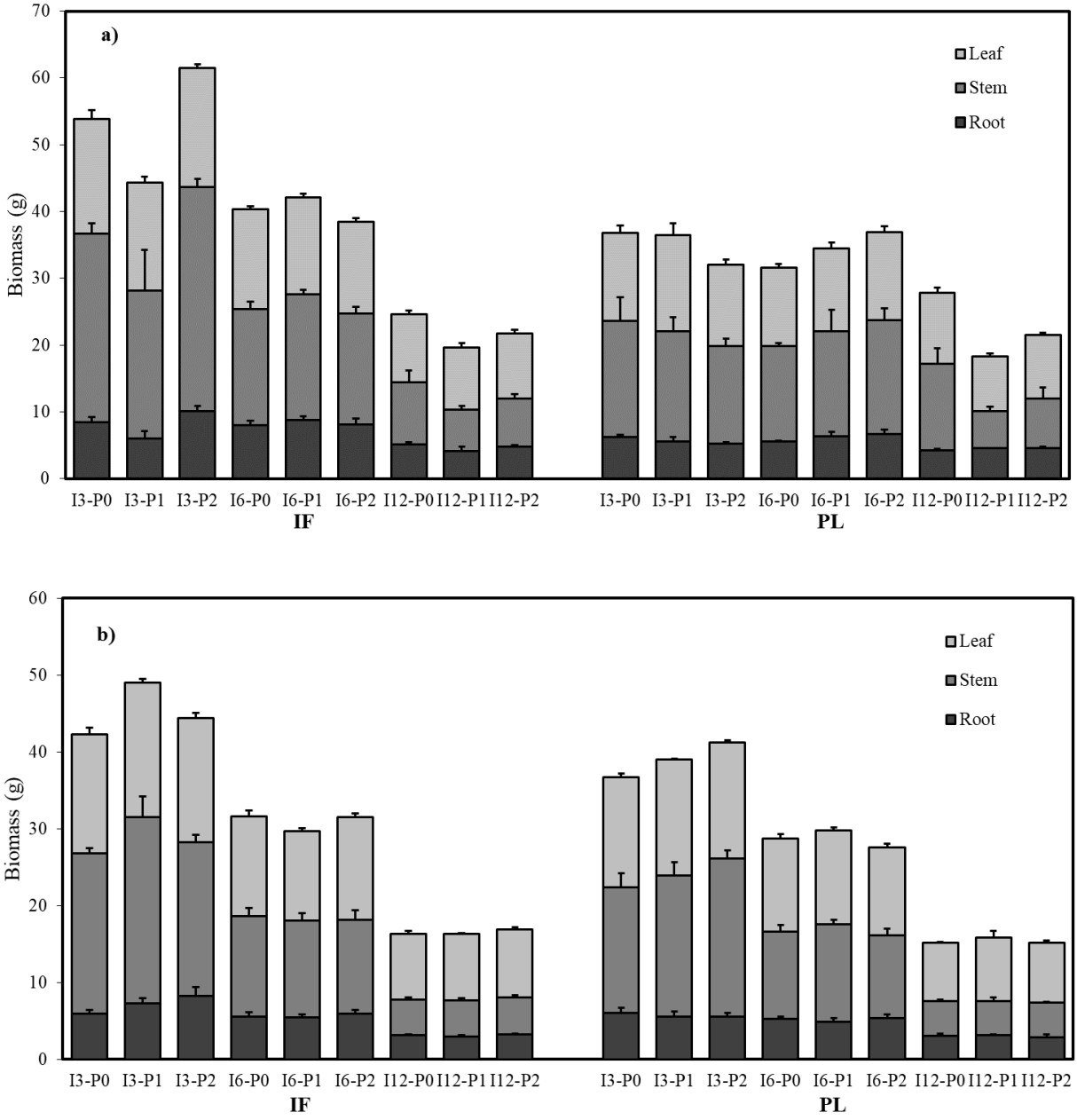

Fig. 1. Response of corn biomass (dry matter basis) to N source, irrigation, and PGPR inoculation at 57 DAP during a) June to September (Exp. 1) and August to October (Exp. 2). Data represent means and standard errors of replicates.