Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system

A.O. Adesemoye, H.A. Torbert, and J.W. Kloepper

Abstract: A 3 year field study was conducted with field corn from 2005 to 2007 to test the hypothesis that microbial inoculants that increase plant growth and yield can enhance nutrient uptake, and thereby remove more nutrients, especially N, P, and K from the field as part of an integrated nutrient management system. The field trial evaluated microbial inoculants, which include a commercially available plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhiza fungi (AMF), and their combination across 2 tillage systems (no-till and conventional till) and 2 fertilization regimes (poultry litter and ammonium nitrate). Data were collected on plant height, yield (dry mass of ears and silage), and nutrient content of corn grain and silage. In addition, nutrient content of soil was determined, and bioavailability of soil nutrient was measured with plant root simulator probes. Results showed that inoculants promoted plant growth and yield. For example, grain yields ($kg \cdot ha^{-1}$) in 2007 for inoculants were 7717 for AMF, 7260 for PGPR+AMF, 7313 for PGPR, 5725 for the control group, and for fertilizer were 7470 for poultry litter and 6537 for NH_4NO_3. Nitrogen content per gram of grain tissues was significantly enhanced in 2006 by inoculant, fertilizer, and their interactions. Significantly higher amounts of N, P, and K were removed from the plots with inoculants, based on total nutrient content of grain per plot. These results supported the overall hypothesis and indicate that application of inoculants can lead to reduction in the build up of N, P, and K in agricultural soils. Further studies should be conducted to combine microbial inoculants with reduced rates of fertilizer.

Key words: plant growth-promoting rhizobacteria, arbuscular mycorrhiza fungi, integrated nutrient management, fertilizer, poultry litter.

Résumé : Une étude de terrain d'une durée de 3 ans a été réalisée sur des champs de maïs entre 2005 et 2007, afin de vérifier l'hypothèse voulant que des inoculants microbiens qui augmentent la croissance des plants et leur récolte puissent augmenter la captation des nutriments, prélevant ainsi plus de nutriments du sol, notamment le N, le P et le K dans le contexte d'un système intégré de gestion des nutriments. L'étude de terrain comportait l'évaluation d'inoculants microbiens, dont des rhizobactéries favorisant la croissance des plants disponibles commercialement, des champignons mycorrhizes arbusculaires et leur combinaison, parallèlement à deux systèmes de labour (sans labour et labour conventionnel) et 2 régimes de fertilisation (fumier de poulet et nitrate d'ammonium). Des données ont été recueillies sur la hauteur des plants, le rendement (masses sec d'épis et de fourrage) et sur le contenu en nutriments de grains de maïs et du fourrage. De plus, le contenu en nutriments du sol a aussi été déterminé et la biodisponibilité des nutriments du sol a été mesurée à l'aide de sondes PRS (« plant root stimulator »). Les résultats ont démontré que les inoculants ont favorisé la croissance et le rendement des plants. Par exemple, en 2007, les rendements en grains ($kg \cdot ha^{-1}$) en présence d'inoculants étaient de 7717 avec les mycorrhizes arbusculaires, 7260 avec des rhizobactéries favorisant la croissance combinées aux mycorrhizes arbusculaires, 7313 avec les rhizobactéries favorisant la croissance comparativement à 5725 pour le groupe contrôle; alors que le rendement des fertilisants était de 7470 avec le fumier de poulet et de 6537 avec le NH_4NO_3. En 2006, le contenu en azote par gramme de tissu de grains était significativement augmenté par les inoculants, les fertilisants et leurs combinaisons. Des quantités significativement plus élevées de N, de P et de K ont été prélevées des parcelles avec inoculants, selon le contenu total en nutriments des grains par parcelle. Ces résultats appuient l'ensemble de nos hypothèses et indiquent que l'application d'inoculants peut conduire à une réduction de l'accumulation de N, de P et de K dans les terres agricoles. D'autres études devraient être réalisées en combinant les inoculants microbiens et des quantités réduites de fertilisants.

Mots-clés : rhizobactéries favorisant la croissance, champignons mycorrhizes arbusculaires, gestion intégrée des nutriments, fertilisant, fumier de poulet.

[Traduit par la Rédaction]

Received 28 April 2008. Revision received 26 July 2008. Accepted 1 August 2008. Published on the NRC Research Press Web site at cjm.nrc.ca on 4 October 2008.

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 doi:10.1139/W08-081

Introduction

Fertilization is an essential practice to optimize crop productivity. However, fertilization has also been associated with nitrate and phosphate contamination of surface and (or) groundwaters, which can be attributed in large part to low efficiency in plant nutrient uptake. Phosphorus (P) is highly reactive with Fe, Al, and Ca, leading to P precipitation at rates up to 90% (Requena et al. 1997; Gyaneshwar et al. 2002; Barlog and Grzebisz 2004), but overapplication of P can result in P runoff causing eutrophication of surface waters. Nitrogen (N) fertilization can also lead to runoff and leaching of nitrate into groundwater. In fact, nitrate leaching has been reported to be inevitable in agriculture production (Ottman and Pope 2000; Steinshamn et al. 2004; Fan et al. 2005; Kleinman et al. 2005; Ohno et al. 2005; Torbert et al. 2005).

Partly as a result of these problems, guidelines for P fertilization have been developed in some regions. For instance, many US states include P source coefficients in site assessment indices so that materials applied to agricultural soils are evaluated on the basis of their relative availability to enrich dissolved reactive P in runoff (Sharpley et al. 2003). Hence, integrated nutrient management (INM) is now being promoted to reduce negative impacts of P and N. The INM system promotes low chemical input but improved nutrient-use efficiency by combining natural and man-made sources of plant nutrients for increased crop productivity in an efficient and environmentally prudent manner that will not sacrifice productivity of future generations (Gruhn et al. 2000).

Free-living plant growth-promoting rhizobacteria (PGPR) have shown promise as biofertilizers (Podile and Kishore 2007). Many previous studies and reviews had reported plant growth promotion, increased yield, solubilization of P or K, uptake of N and some other elements through inoculation with PGPR (de Freitas et al. 1997; Rodriguez and Fraga 1999; Joo et al. 2004; Sheng and He 2006; Glick et al. 2007). In addition, some studies have shown that treatment with PGPR enhances root growth, leading to a root system with large surface area and increased number of root hairs (Mahaffee and Kloepper 1994; Mantelin and Touraine 2004). Although, PGPR may be helpful in INM, they have not been evaluated as components of INM systems. Arbuscular mycorrhiza fungi (AMF) are another group of microbial inoculants that can influence plant growth and water and nutrient uptake. Extraradical hyphae of AMF act as a bridge between the soil and plant roots; however, AMF effectiveness is affected by soil P concentration (Liu et al. 2000; Bianciotto and Bonfante 2002; Stewart et al. 2005).

Our overall hypothesis was that microbial inoculants that increase plant growth and yield can enhance nutrient uptake and thereby remove more nutrients, especially N, P, and K from the field as part of an INM system. In this study, we investigated PGPR, AMF, and their combination, as the microbial inoculants, for effects on growth and nutrition of corn grown in a long-term field study under 2 tillage systems (no-till and conventional till) and 2 fertilization regimes (poultry litter and ammonium nitrate).

Materials and methods

Experimental design

The experimental design was a split–split plot in a randomized complete block with 4 replications. The main plot consisted of 2 tillage types (conventional till (CT) and no-till (NT)), 2 subplots of either chemical fertilizer or manure (poultry litter), and sub-subplots consisting of 4 types of inoculants (PGPR, a mixture of PGPR and AMF, AMF, and a water control). Each of the final sub-subplots was 7.6 m (25 ft) long by 0.9 m (3 ft) wide. All treatments were applied to the same plots from year to year to confine treatment effects.

Field preparation and fertilizer application

This study was conducted on continuous corn plots within an existing long-term crop rotation field situated at the Sand Mountain Research and Education Center of the Alabama Agriculture Experiment Station in Crossville, Alabama. The initial split-plot had been in place for 25 years before the introduction of an additional split by 2005. Thus, the study period for this report spanned the summers of 2005, 2006, and 2007. We report here the results for 2006 and 2007. The test crop was field corn (CroplanTR1167RR), and seeding was done each year in April, with the specific date depending on weather conditions each year. The plots for CT were prepared by shallow disking, resulting in incorporation of crop residues, while NT plots were planted by a NT planter. The manure used was dried poultry litter, applied at the rate of 427.5 kg·ha^{-1}. At planting, crops received 57 kg N·ha^{-1} as ammonium nitrate (NH_4NO_3, 32% N) and 171 kg P·ha^{-1} as triple superphosphate. They were then side-dressed with 171 kg N·ha^{-1} as NH_4NO_3 between 4 and 5 weeks after planting. Also, 120.8 kg·ha^{-1} of N:P:K at a ratio of 0:0:48, 22.8 kg·ha^{-1} of S, and 114 kg·ha^{-1} of lime were applied based on the recommendations of Auburn University Soil Testing Laboratory, and no micronutrients were added.

Application of microbial inoculants—PGPR and AMF

One commercially available microbial PGPR and one AMF product were selected as models for the study. The selected PGPR product was Plant Growth Activator (PGA) (Organica, Norristown, Pennsylvania), while the AMF product was *Glomus intraradices* (Becker Underwood, Ames, Iowa). The PGA is a mixture of many *Bacillus* sp. strains and was prepared at the label rate of 1 tablespoon per gallon (1 tablespoon = 15 cm^3; 1 gallon = 3.785 411 784 dm^3) of water. The suspensions of both PGA and AMF were applied according to manufacturer's recommendation, around the base of each growing seedling at 2 weeks after seeding. In plots receiving single inoculant treatment, 100 mL suspension of the appropriate inoculant was applied per plant. For the plots receiving co-inoculation of PGPR and AMF, 50 mL suspensions of each inoculant were applied per plant. Controls were treated with 100 mL of water per plant.

Plant root simulator probes

Plant root simulator probes (PRS) (Western Ag Innovations Inc., Saskatoon, Saskatchewan, Canada) were buried in the plots. The probes estimate nutrient bioavailability by measuring nutrient supply rate through an ion exchange

resin (IER). The probes are designed to be susceptible to all edaphic factors affecting nutrient uptake by plants, so that they mimic plant roots (Hangs et al. 2004). The pattern of nutrient availability over time was monitored, and the supply rate to the probe was compared with nutrient uptake in plants. The probes were used in pairs—one for anion exchange (orange color) and the other for cation exchange (purple color). The first set of probes was removed 24 h after burial. Subsequent burials were made into the same location, and the probes were inserted for 2 week intervals before removal. On each subplot, 2 pairs of the probes were installed. After being removed from the soil, probes were washed thoroughly with deionized water and placed in plastic bags under moist and cold conditions on ice for transporting to the laboratory. They were later sent to Western Ag Innovations Inc. for analysis. The details about washing and preparing the probes in the laboratory, including analysis procedures, were previously described by Hangs et al. (2004).

Monitoring plant growth, harvesting, and estimation of yield

Plants within the middle 150 cm of each plot were chosen for data collection to avoid edge effects. Plant height was measured at about 8 weeks after planting (V7–8 growth stage). At physiological maturity (R6), destructive harvesting was done. Ears (cob plus grains) of corn within the middle 150 cm of each sub-subplot were harvested from the stalk. Masses of ears were recorded in the field. Corn stalks were cut near the ground (at the crown of the roots), and total fresh mass of stalks from each sub-subplot was recorded. The stalks were shredded with a chipper shredder (Briggs and Stratton, Wauwatosa, Wisconsin), after which a subsample was taken at random from the silage, packed into a small bag, and weighed. Samples from all plots were then transported to the USDA–Agricultural Research Services, National Soil Dynamics Laboratory (USDA–ARS–NSDL) in Auburn for drying and further processing. Drying was done at 55 °C for 2 weeks, and dry masses of ears and silage were recorded. Ears were shelled with locally fabricated equipment to remove seeds, which were then weighed. The seeds were ground with a Wiley Mill model No. 4 (Arthur Thomas Scientific, Swedesboro, New Jersey) and further grinding was done with a Cyclone Sample Mill (Udy Corporation, Fort Collins, Colorado) to achieve a fine powder for nutrient analysis. Both mills were used for grinding the silage.

Nutrient content of plant tissues and soil

Tissues of ear and silage samples were ashed to analyze their nutrient contents. The samples were analyzed for N and C using TruSpec CN (LECO, St. Joseph, Michigan). Analyses for other elements, including P, K, S, Ca, Mg, Zn, Cu, Mn, and Fe, were done at the Soil Testing Laboratory, Auburn University, using inductively coupled plasma – atomic emission spectroscopy (ICP–AES) (Varian, Victoria, Australia). Only the steps involved in preparing the samples for analysis based on the procedure developed by Teem (1986) will be reported here because both ICP–AES and TruSpec CN are automated systems. For each sample, approximately 0.5 g of the dry fine powder (which can pass a 40 mesh, i.e., 0.60 mm stainless steel sieve) was placed into a 50 mL beaker, covered with a watch glass, and placed in a muffle furnace. After heating to 450 °C for 4 h, 10 mL of 1 mol·L^{-1} HNO_3 was added to the grayish-colored ash and slowly evaporated to dryness on a hot plate, ensuring that it did not bake. Subsequently, 10 mL of 1 mol·L^{-1} HCl was added to dissolve the residue. It was warmed nearly to boiling and transferred to a 100 mL volumetric flask. The beaker was washed 3 times with small amounts of water, and the volume was made up to 100 mL followed by filtration. The elemental composition of the filtrate was then determined using ICP–AES. Nutrient uptake on a per plot basis was estimated through uptake per gram of plant tissue multiplied by total yield per plot (i.e., yield × percent nutrient per gram of plant tissue).

Soil samples were collected from the plots, close to the plant roots but not the rhizosphere, and were analysed at the start and the end of the 3 year study period to detect any changes. Mehlich 1 (double acid) extraction method, common in the southeastern USA (Mehlich 1953), was used for soil analysis in which 5 g of sieved air-dried soil was added to a 150 mL extraction flask, followed by 25 mL of Mehlich 1 extracting solution (0.05 mol·L^{-1} H_2SO_4 + 0.05 mol·L^{-1} HCl) and then shaken for 5 min on a reciprocating shaker (Barnstead/Thermolyne, Dubuque, Iowa) at 180 oscillations·min^{-1}. It was centrifuged (International Equipment Co, Needham, Massachusetts) at 80% speed for 10 min, filtered through a Whatman No. 2 filter paper, and analyzed using ICP–AES.

Data analysis

Data for plant growth, yield, and nutrient uptake were analyzed using the mixed procedure of the Statistical Analysis System (SAS) 9.1 (SAS Institute Inc., Cary, North Carolina). Mixed procedure was recommended for designs such as split–split plot randomized complete block due to the method used in fitting linear mixed models, including its ability to apply likelihood methods to complex mixed models (Littell et al. 2006). Slices test was done to determine the equality of simple effects of factors for each level of other factors. Pairwise comparisons of the least square means were obtained with the 'Diff' option, while the 'adjust = sim' option provided a family-wise error rate protection. Analysis of variance (ANOVA) was based on each year to allow yearly comparisons and to avoid introduction of another factor (year), which could violate the requirement of independence of the residuals. Treatment effects and the interactions among treatments were tested. The Glimmix procedure was used to plot diffograms (mean–mean scatter plot) (Littell et al. 2006). Gplot and Boxplot procedures were used for the data on soil nutrient content and PRS probes, respectively. Unless otherwise stated, statistical significance was considered at $\alpha = 0.05$.

Results

Growth and yield promotion

Inoculation of AMF, PGPR, and the combination of the 2 (PGPR+AMF) resulted in significantly greater plant height compared with the noninoculated control (Fig. 1). The mean height of plants in each of the 3 inoculants was not differ-

Fig. 1. Plant height for 2006 and 2007. AMF, arbuscular mycorrhiza fungi; PGPR, plant growth-promoting rhizobacteria; PG+AM, co-inoculation of AMF and PGPR; WTR, water (no inoculation); PL, poultry litter; and NH_4, ammonium nitrate.

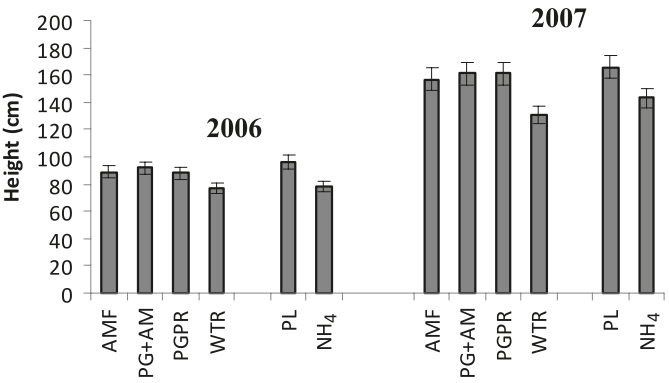
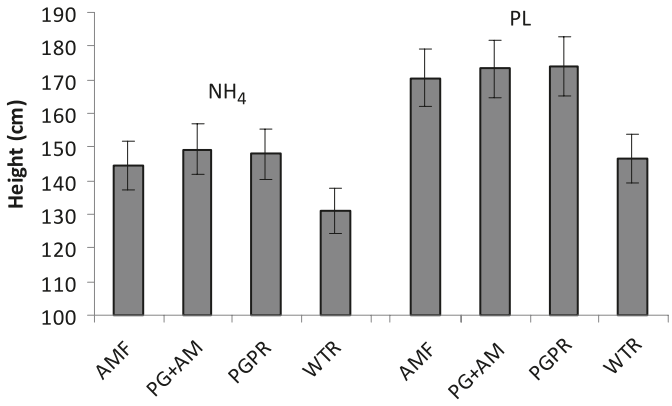


Fig. 2. Significant interactions between inoculant and fertilizer on plant height in 2006. AMF, arbuscular mycorrhiza fungi; PGPR, plant growth-promoting rhizobacteria; PG+AM, co-inoculation of AMF and PGPR; WTR, water (no inoculation); NH_4, ammonium nitrate; and PL, poultry litter.



ent. Comparing the 2 fertilizers, plants from plots that received poultry litter were taller than those from plots treated with ammonium nitrate. Tillage effect was not significant. The overall growth across all treatments in 2006 was greater than 2007 due to the severe drought in Alabama in 2007; however, the trend of the treatment effects was generally similar.

There was a significant interaction effect among inoculant and fertilizer in 2006 (Fig. 2). Height of plants on plots that received inoculants within plots of ammonium nitrate was greater than plants that received no inoculant. A similar trend was observed for inoculants on plots with poultry litter. All plots with inoculants within the poultry litter treatment showed relatively greater height than those of their corresponding inoculants within ammonium nitrate treatments.

Analysis showed that fertilization and inoculation affected corn yields (including grain and silage) significantly, but tillage did not affect yield (Table 1). For both grain and silage, plants from plots that received poultry litter yielded more than those that received ammonium nitrate (Table 1). A comparison of grain yield among different inoculant treatments in 2006 and 2007 revealed that the yields for both PGPR and AMF were similar to each other, but all were

Fig. 3. Diffogram (mean–mean scatter plot) comparing the effect of inoculant on grain yield in 2007 (A) and 2006 (B). F, arbuscular mycorrhiza fungi; P, plant growth-promoting rhizobacteria (PGPR); M, co-inoculation of AMF and PGPR; and W, water (no inoculation). Yield is measured in kilograms per hectare. The 45° reference line indicates whether 2 least-square means are significantly different at a significance level of 0.05. The thick lines drawn at the intersection of grid lines corresponds to $(1 - \alpha) \times 100\%$ confidence interval of the difference of the 2 least-square means in each comparison. Any thick line that crosses the 45° reference line implies no significant difference for that comparison.

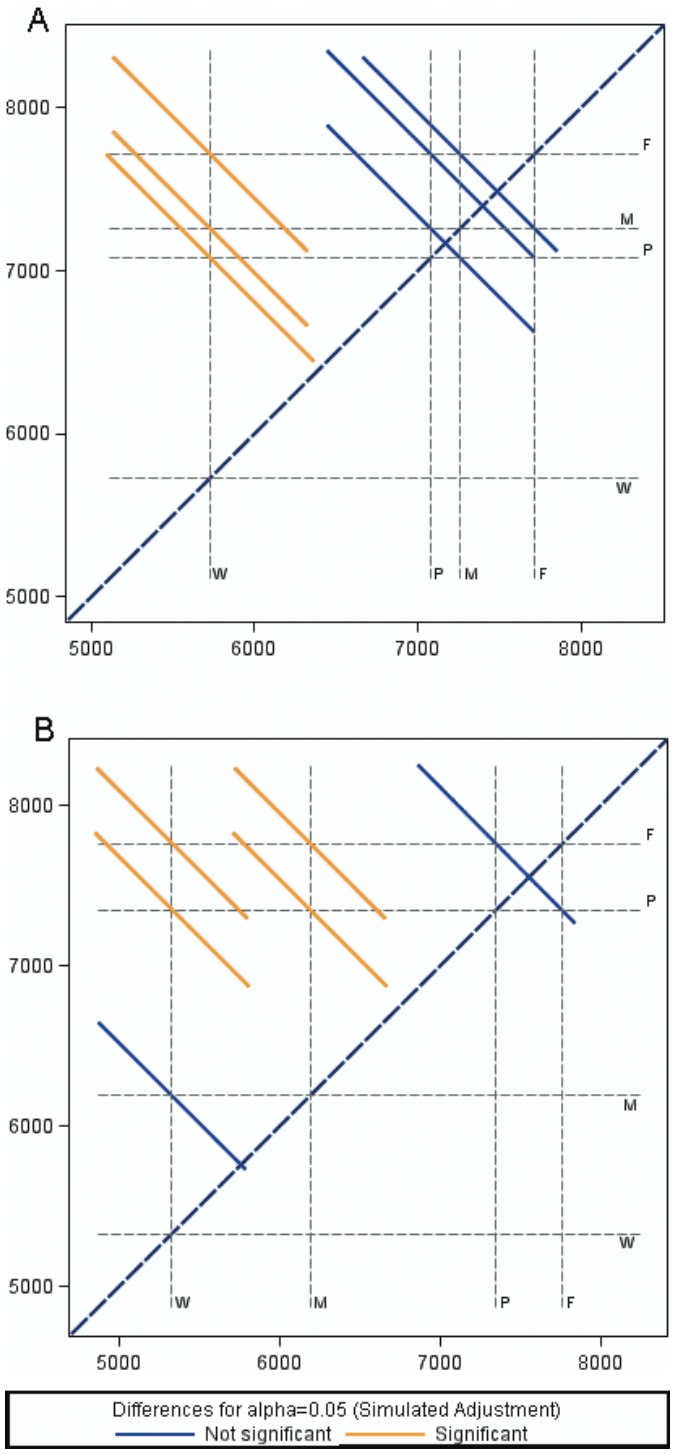


Fig. 4. Significant interactions between inoculant and tillage on grain yield in 2007. AMF, arbuscular mycorrhiza fungi; PGPR, plant growth-promoting rhizobacteria; PG+AM, co-inoculation of AMF and PGPR; WTR, water (no inoculation); CT, conventional till; and NT, no-till.

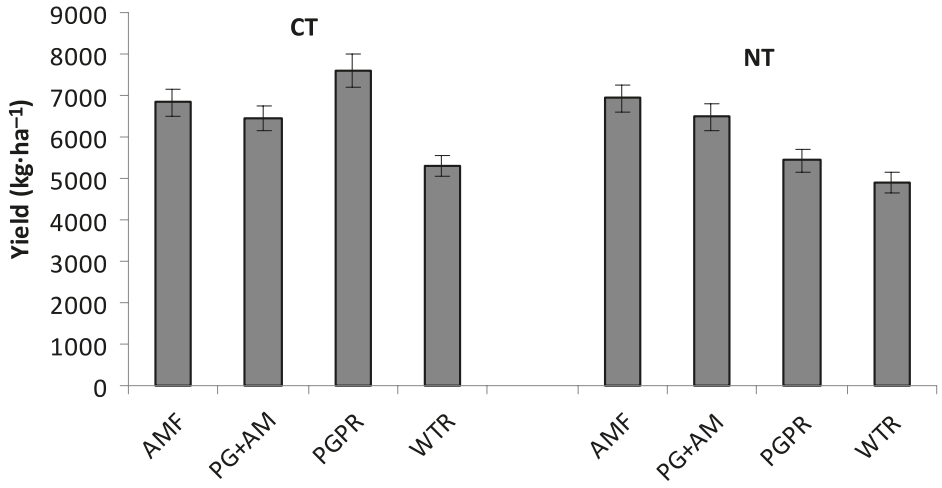


Table 1. (A) Mean yield and (B) ANOVA for yield in 2007.

(A) Mean yield (kg·ha^{-1})		
Treatment	Grain[a]	Silage[a]
Inoculants		
AMF	7717	8994
PGPR+AMF	7260	8534
PGPR	7313	8517
WTR	5725	6671
Fertilizers		
PL	7470	8751
NH_4	6537	7607

(B) Analysis of variance (ANOVA)

		Grain		Silage	
Treatment	df	*F*	Pr > F	*F*	Pr > F
Tillage (T)	1	2.5	0.21	0.14	0.73
Fertilizer (F)	1	15.59	0.03	23.3	0.02
Inoculant (I)	3	8.25	0.001	5.33	0.008
T*F	1	0.17	0.71	0.91	0.41
T*I	3	3.10	0.05	0.73	0.55
T*F*I	3	0.76	0.53	0.85	0.49

Note: Four types of inoculants and 2 types of fertilizers include the following: AMF, arbuscular mycorrhiza fungi; PGPR, plant growth-promoting rhizobacteria; PGPR+AMF, co-inoculation of AMF and PGPR; WTR, water (no inoculation); PL, poultry litter; and NH_4, ammonium nitrate.

[a]Grain and silage are mean yield (kg·ha^{-1}) of corn grain and silage.

generally greater than the noninoculated plots. It is interesting that despite the drought in 2007, inoculants still produced better yield (Fig. 3) than the noninoculated control. Although the effect of tillage alone was not significant, there was a significant interaction effect of inoculant by tillage (Fig. 4).

Nutrient content of PRS probes

Figure 5 presents the fluctuations of nutrients over time in the plots in 2006. The graph was plotted only for the interaction of tillage and fertilizer without any specificity for inoculants. It was designed to measure available nutrient as a base for a comparison of the effect of inoculants on plant nutrient uptake. The interactions between tillage and fertilizer types included (*i*) conventional tillage with poultry litter (CTL), (*ii*) conventional tillage with ammonium nitrate (NH_4NO_3) (CTO), (*iii*) no-till with poultry litter (NTL), and (*iv*) no-till with NH_4NO_3 (NTO). It became clear that through most of the growing season, more P was available for plant use in the NTL plots. For N, higher bioavailability was more often observed in NTO plots. For potassium (K), the highest bioavailability was between CTL and NTL plots. The fluctuations in nutrient availability during the growing season and the decreases towards the end of the growing season are similar to the results observed by Galvez et al. (2001).

Nutrient content of soil

Soil analysis showed that the amount of nitrogen in the field increased at the end of the study in 2007 compared with 2005. The trend was the same across all treatments listed above. As the amount of nitrogen increased, the variance and standard deviation decreased (Fig. 6A). With P, however, significant increases were observed in NTL and CTL plots, but not in NTO and CTO plots (Fig. 6B). For K, a significant increase was observed only in CTO plots (Fig. 6C).

Nutrient contents of plant samples

Inoculant and fertilizer (Fig. 7) as well as their interaction (Fig. 8) significantly increased N content per gram of grain tissues in 2006 but not in 2007. Also, the enhancement of nutrient uptake per gram of plant tissues was not consistent across all treatments for the 2 years. Fertilizer treatment affected phosphorus uptake, but inoculant alone had no significant effect per gram of tissue. Specifically, in 2006 *P* values for analyzed phosphorus data were 0.003 for fertilizer, 0.2 for inoculant, 0.009 for fertilizer by inoculant interaction in grain, but were 0.03, 0.36, and 0.37, respectively, in silage. Treatment effects on nutrient uptake per gram of plant tissue could possibly be more consistent at other

Fig. 5. Supply rate (bioavailability) of N (A), P (B), and K (C) in the plots in 2006. The plots were as follows: NTO, no-till with ammonium nitrate; NTL, no-till with poultry litter; CTO, conventional till with ammonium nitrate; and CTL, conventional till with poultry litter.

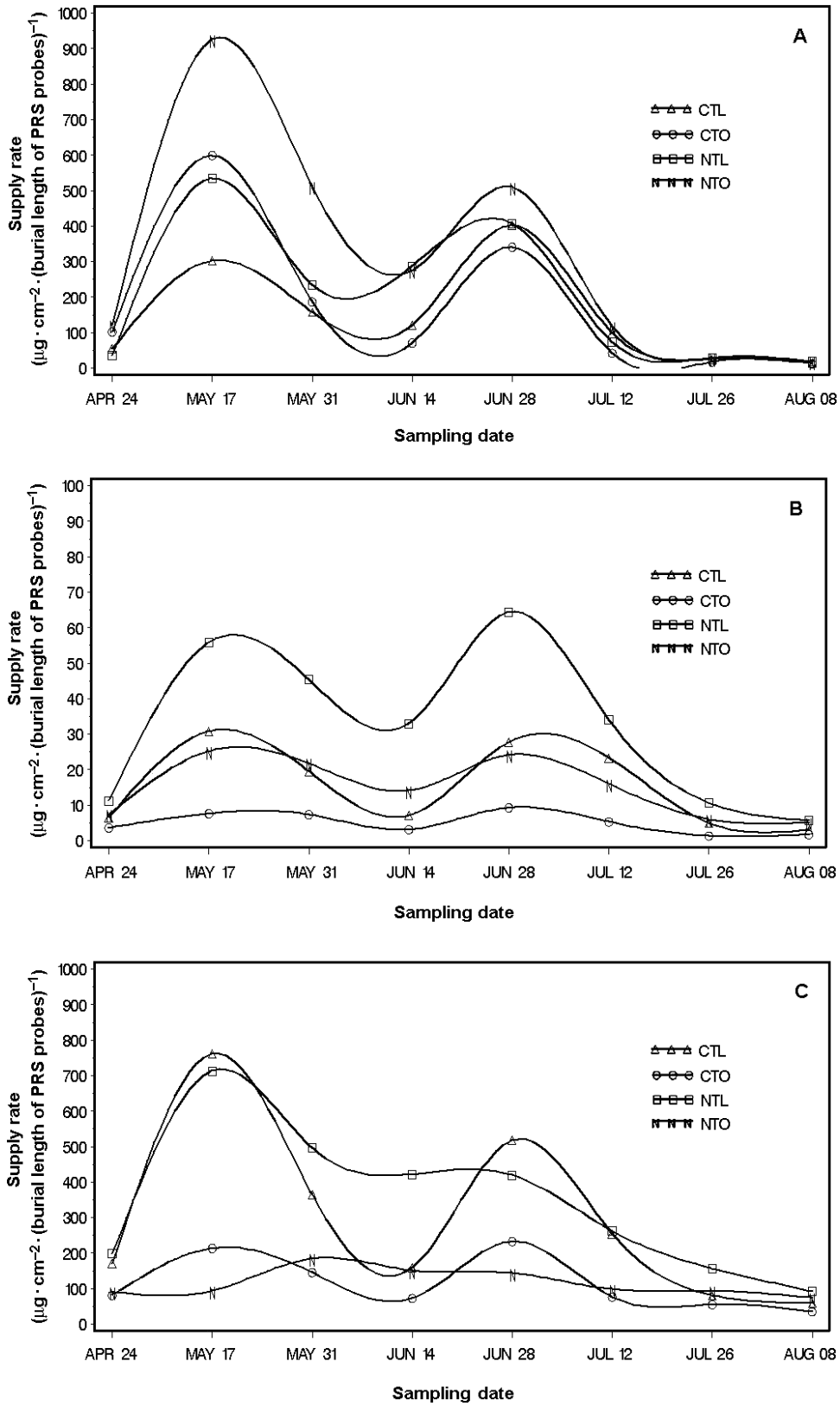


Fig. 6. Content of N (A), P (B), and K (C) in soil before and after the study. NTO, no-till with ammonium nitrate; NTL, no-till with poultry litter; CTO, conventional till with ammonium nitrate; and CTL, conventional till with poultry litter. Block number 1 represents 2005 and block number 2 represents 2007.

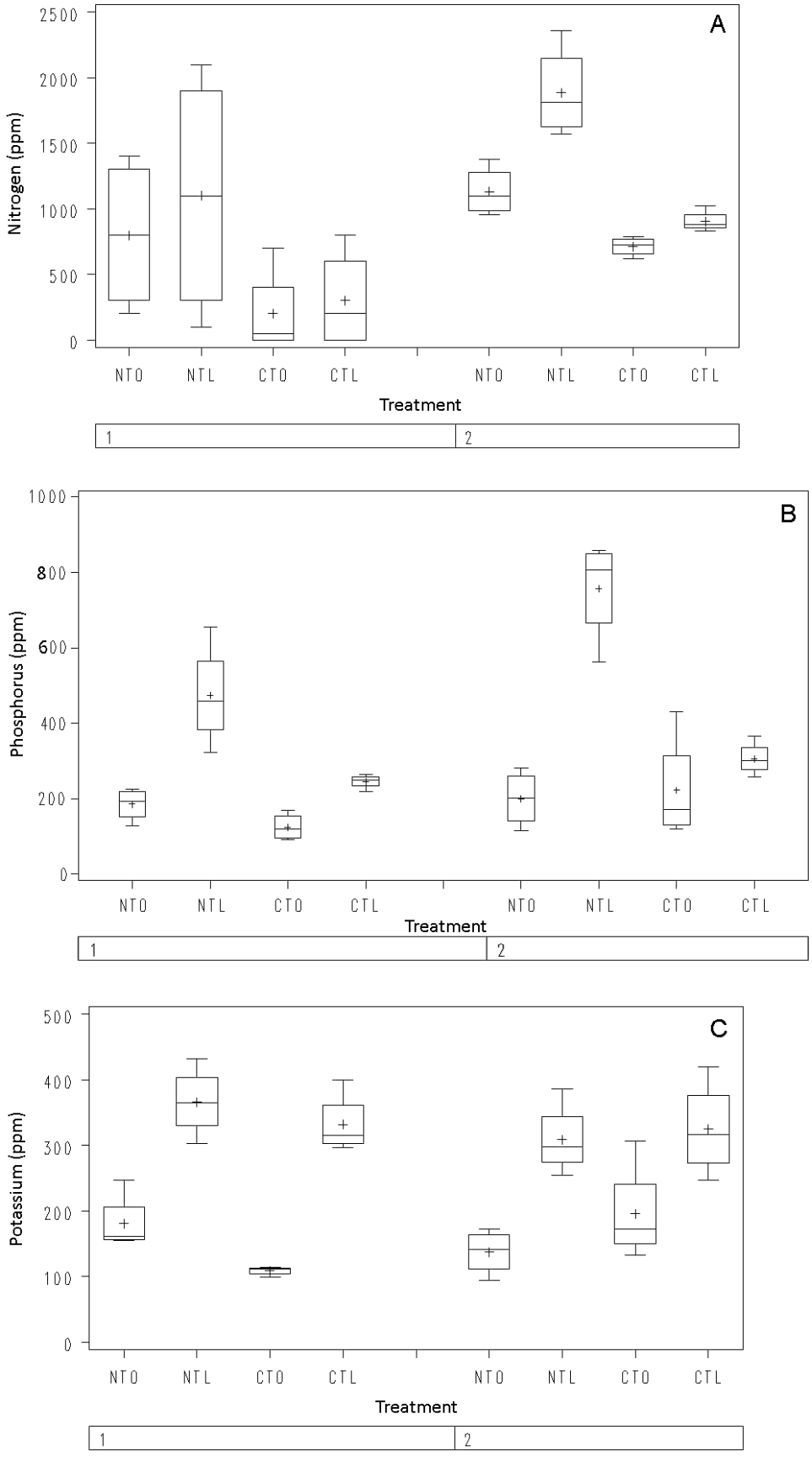


Fig. 7. Nitrogen content per gram of grain tissues for 2006. AMF, arbuscular mycorrhiza fungi; PGPR, plant growth-promoting rhizobacteria; PG+AM, co-inoculation of AMF and PGPR; WTR, water (no inoculation); PL, poultry litter; and NH_4, ammonium nitrate.

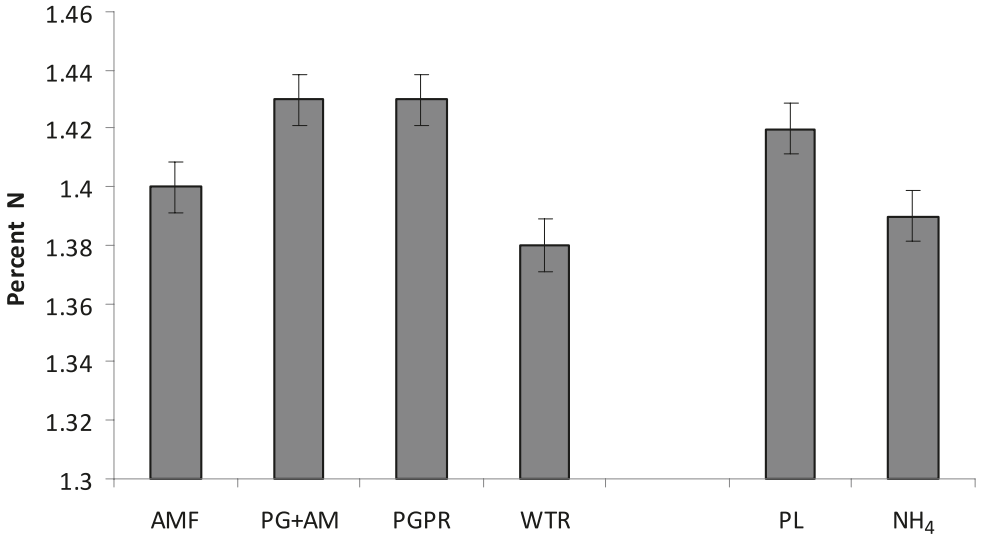
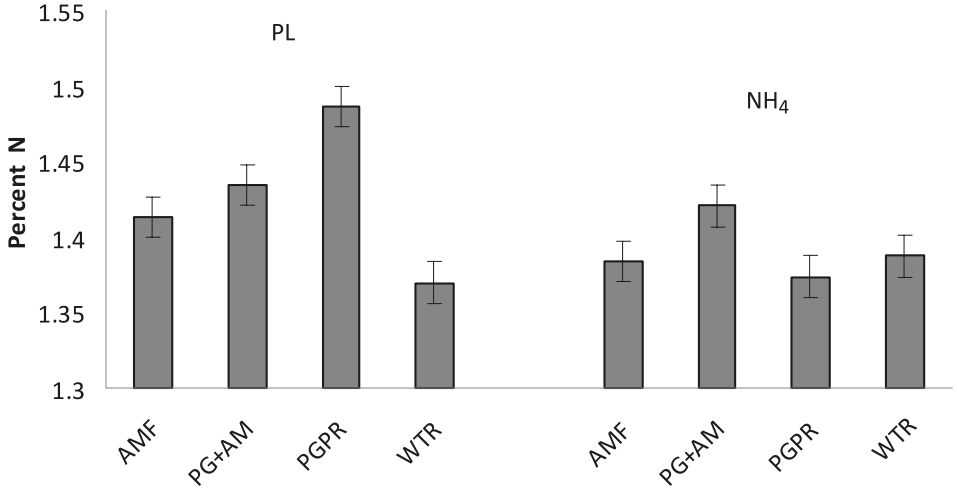


Fig. 8. Interactions of inoculant and fertilizer for nitrogen per gram of grain tissue in 2006. AMF, arbuscular mycorrhiza fungi; PGPR, plant growth-promoting rhizobacteria; PG+AM, co-inoculation of AMF and PGPR; WTR, water (no inoculation); PL, poultry litter; and NH_4, ammonium nitrate.



growth stages besides the physiological maturity stage in which samples were taken for nutrient analysis. However, we had chosen only this maturity stage for nutrient content evaluation because that is the stage that could best reflect the amount of nutrients removed from the field through harvesting of plants.

The interaction effect of inoculant and fertilizer was significant on K uptake per gram of silage tissue (Fig. 9). In 2006, poultry litter interactions with inoculants significantly enhanced uptake of K in corn silage compared with ammonium nitrate interactions with inoculants. Overall, nutrient uptake (N, P, and K) in grain per plot was significantly higher for all inoculated plots (Table 2). Our focus in this report is on the 3 most important or limiting elements: N, P, and K. However, it is pertinent to mention that we also observed significant inoculant effects on some of the other elements, and we have shown magnesium as an example in Table 2.

Discussion

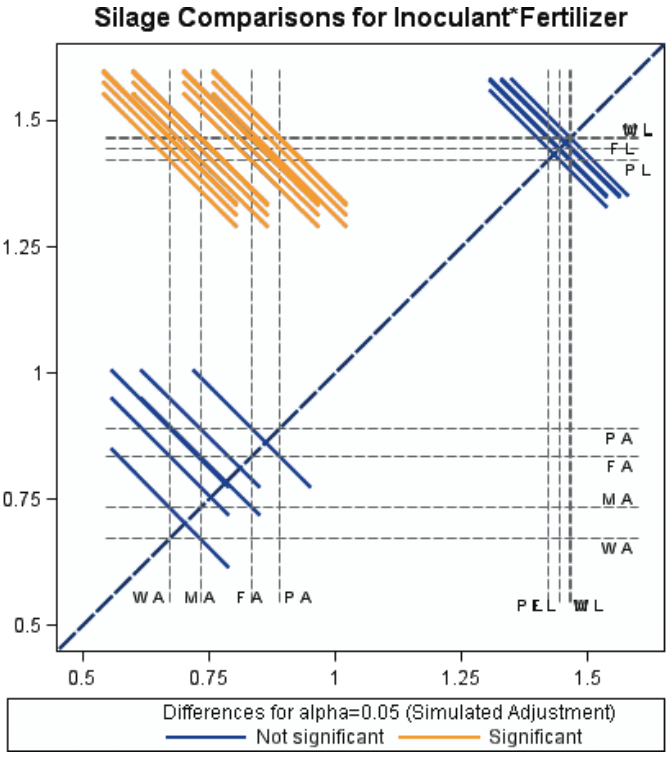
Our results demonstrate that microbial inoculants can increase nutrient content of plants and overall plant growth. For example, treatment with inoculants resulted in increased N per gram of seed and N uptake per plot (Figs. 7 and 8). The use of inoculants for enhanced N uptake could therefore be applied to improve N uptake efficiency and potentially reduce nitrate leaching. Also, more P was removed from the plots that received inoculants, indicating that the uptake efficiency of P was also improved and likewise could reduce potential losses of P to the environment (Table 2). Hence, inoculants have potential as inputs in integrated nutrient management systems to help reduce build up, leaching, or runoff of nutrients from fields. Treatment effects of inoculants on N and K uptake per gram of plant tissue was more strongly expressed in the 2006 than in the 2007 growing season, and this may be related to the drought in 2007. It

Table 2. Estimated total nutrient uptake per plot in 2007.

	Nitrogen		Phosphorus		Potassium		Magnesium	
Treatment	Grain	Silage	Grain	Silage	Grain	Silage	Grain	Silage
AMF	9929a	8199a	2424a	2888a	3310a	14843a	888a	2516a
PGPR+AMF	9002a	6665ab	2329a	2318a	3189a	13481ab	878a	2345ab
PGPR	9272a	6532b	2331a	2784a	3159a	13194ab	891a	2397ab
WTR	7401b	5615b	1948b	1959a	2646b	11122b	725b	1985b

Note: Values in each column with different letters are significantly different at $P = 0.05$. AMF, arbuscular mycorrhiza fungi; PGPR, plant growth-promoting rhizobacteria; PGPR+AMF, co-inoculation of AMF and PGPR; WTR, water (no inoculation).

Fig. 9. Diffogram (mean–mean scatter plot) of the interaction of inoculant and fertilizer for potassium content in silage in 2006. A, ammonium nitrate; L, poultry litter; F, arbuscular mycorrhiza fungi; P, PGPR; M, AMF+PGPR; and W, water. Potassium content is measured in percent. The 45° reference line indicates whether 2 least-square means are significantly different at a significance level of 0.05. The thick lines drawn at the intersection of grid lines correspond to $(1 - \alpha) \times 100\%$ confidence interval of the difference of the 2 least-square means in each comparison. Any thick line that crosses the 45° reference line implies no significant difference for that comparison.



was obvious from the results that the soil and the general environmental conditions have impacts on the efficacy of PGPR and AMF.

Looking at the total uptake of each element on the basis of total nutrient content in grain per plot, significantly higher amounts of N, P, and K were removed from those plots that received inoculants compared with the control (Table 2). This enhancement of nutrient uptake in plant tissues per plot due to inoculant becomes clearer by observing the effects as being dependent on plant development rather than as an uptake function (de Freitas et al. 1997; Mantelin and Touraine 2004). This finding suggests that enhanced plant growth with better root development gives the potential for greater nutrient uptake.

In our study, promotion of plant growth and yield was achieved by each inoculant and their combination in the 2 years reported (Fig. 2). This finding is in agreement with some previous studies (Mahaffee and Kloepper 1994; de Freitas et al. 1997; Kim et al. 1997; Kloepper et al. 2007). The results of the current study extend the previous findings by the integration of multiple factors (tillage, fertilizers, and inoculants). Contrary to previous reports (Singh and Kapoor 1998), in our study co-inoculation of PGPR and AMF did not produce synergistic effects under field conditions. Plants that received co-inoculation of PGPR and AMF showed virtually the same growth and yield compared with either inoculant alone (Fig. 1 and Table 1). Nonetheless, interactions could exist with different specific PGPR strains and AMF isolates, and the combinations of PGPR and AMF for nutrient management should be further explored.

Generally, our results supported the overall hypothesis that microbial inoculants that increase plant growth and yield can enhance nutrient uptake and thereby remove more nutrients, especially N, P, and K, from the field as part of an INM system. The explanation of Sheng and He (2006) might be the reason for the enhanced uptake of K by microbial inoculant that was observed in our study. They explained that organic acids, e.g., citric, oxalic, tartaric, succinic, and α-ketogluconic, produced by the PGPR *Bacillus edaphicus* strains NBT and its mutants are able to chelate metals and mobilize K from K-containing minerals. For P, treatment effects of inoculants on uptake per gram of plant tissue were not significant despite increased growth, yield, and P removal per plot. These results are similar to that of de Freitas et al. (1997), who reported that some of the PGPR strains significantly increased plant height or pod yield in canola but did not increase P uptake in the seed.

The variation in results for P uptake in our study is consistent with previous reports on N and P in different cropping systems. Two studies from different groups that worked with inoculants (*Azospirillum* strains) reported different results with explanations. Dobbelaere et al. (2002) reported nonsignificant treatment effects on N content of straw and grain in most conditions for wheat and maize, while Saubidet et al. (2002) reported improved uptake of inorganic N in wheat. Combining 3 tillage types, 2 farming systems, and mycorrhiza resident in the field, Galvez et al. (2001) showed that treatment effects on nutrient uptake in corn (maize) depended on growth stage. For instance, N concentrations in corn shoots were greater in plants grown under

low input than under conventional agriculture at the 8 leaf (V8) stage, but the opposite occurred at the dough (R4) stage. Additionally, they observed higher P concentration in shoot for conventional than for low input farming at the vegetative stages and higher in no-till than in tilled soil at all stages of growth, but that did not translate into increased growth and yield. They explained that the high P content of the soil limited the benefit on the resident mycorrhiza population, which increased the influence of what they described as yield-depressing factors.

The information on soil nutrient content at the start and the end of this study for tillage and fertilizer combination without inoculants (Fig. 6) presents a model for how nutrients could build up in a long-term fertilization, as previously explained by Sharpley et al. (2003). Following the inclusion of inoculants (PGPR and (or) AMF), more nutrient uptake per plot was observed, which could lead to a reduction in nutrient build up. Removal of those crops that had enhanced capacity at the end of the growing season would be the best step in practically reducing nutrient build-up from fertilizers. Without removing the plants, plant nutrient may get back into the biogeochemical cycle through decomposition.

Findings on bio-available nutrients as indicated by the PRS probes (Fig. 5) show that with tillage and fertilizer alone (without their interaction with inoculants), there was hardly any difference towards the end of the growing season. Earlier in the season, there were more available nutrients in poultry litter plots than in inorganic fertilizer, but conventional till and no-till were not significantly different. Considering the advantages observed by Wood and Edwards (1992), particularly the cost of machinery, it is expedient to choose no-till over conventional till. Although a combination of no-till with poultry litter (NTL) tends to show more bioavailability of nutrient, it is important to note that the treatment effect of the tillage by fertilizer interaction differs from one element to the other. One pertinent question is whether the difference in bioavailability of nutrients early in the season as indicated by the PRS probes is equivalent to uptake by the plants in the absence of inoculants.

Mahaffee and Kloepper (1994) expressed the need to develop technologies and methodologies that address the problems associated with sustainable agriculture while achieving increased production above current levels to meet the needs of the ever growing population. Based on our results, the combination of no-till, poultry litter, and inoculants (PGPR) is promising for integrated nutrient management. The contribution of a farming system, which integrates multiple factors to improve nutrient use efficiency in a sustainable way, could be viewed from 2 perspectives. First, integrating crop production with livestock wastes offers one way to manage the wastes and maintain high crop productivity at the same time. Second, improved nutrient utilization efficiency from agrochemicals through PGPR and (or) AMF can contribute to the protection of water resources against agro-pollution and reduce the growing cost of fertilizers. Given the enormity of fertility issues in agricultural sustainability, more studies should focus on microbial technologies as means of managing soil nutrients and fertilizer use.

Acknowledgement

The authors are grateful to Dr. Edward van Santen of the Department of Agronomy and Soils, Auburn University, for his advice and help with data analysis.

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