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MINI-REVIEW

Plant–microbes interactions in enhanced fertilizer-use efficiency

Anthony O. Adesemoye · Joseph W. Kloepper

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Abstract The continued use of chemical fertilizers and manures for enhanced soil fertility and crop productivity often results in unexpected harmful environmental effects, including leaching of nitrate into ground water, surface run-off of phosphorus and nitrogen run-off, and eutrophication of aquatic ecosystems. Integrated nutrient management systems are needed to maintain agricultural productivity and protect the environment. Microbial inoculants are promising components of such management systems. This review is a critical summary of the efforts in using microbial inoculants, including plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi for increasing the use efficiency of fertilizers. Studies with microbial inoculants and nutrients have demonstrated that some inoculants can improve plant uptake of nutrients and thereby increase the use efficiency of applied chemical fertilizers and manures. These proofs of concept studies will serve as the basis for vigorous future research into integrated nutrient management in agriculture.

Keywords Plant-microbe interaction · Plant growth-promoting rhizobacteria · Arbuscular mycorrhizal fungi · Integrated nutrient management · Fertilizers

A. O. Adesemoye · J. W. Kloepper
Department of Entomology and Plant Pathology, Auburn University,
209 Life Science Building,
Auburn, AL 36849, USA

Present Address:
A. O. Adesemoye (✉)
Department of Plant Pathology, Michigan State University,
107 Center for Integrated Plant Systems,
East Lansing, MI 48824, USA
e-mail: adeseao@msu.edu

Introduction

The use of fertilizers, including chemical fertilizers and manures, to enhance soil fertility and crop productivity has often negatively affected the complex system of the biogeochemical cycles (Perrott et al. 1992; Steinshamn et al. 2004). Fertilizer use has caused leaching and run-off of nutrients, especially nitrogen (N) and phosphorus (P), leading to environmental degradation (Tilman 1998; Gyaneshwar et al. 2002). Important reasons for these problems are low use efficiency of fertilizers and the continuous long-term use. Despite the negative environmental effects, the total amount of fertilizers used worldwide is projected to increase with the growing world population due to the need to produce more food through intensive agriculture that requires large quantities of fertilizer (Vitousek et al. 1997; Frink et al. 1999).

In the last five decades, the rate of nitrogen, phosphorus, and potassium (NPK) fertilizer application has increased tremendously. The International Fertilizer Industry Association reported that the three countries with the highest fertilizer use in 2006 were China, India, and USA, consuming 50.15, 21.65, and 20.83 million tons of NPK fertilizer, respectively, compared with consumption in 1961 of 1.01, 0.42, and 7.88 million tons, respectively (http://www.fertilizer.org/ifa). The challenge therefore is to continue agricultural productivity in a way that minimizes harmful environmental effects of fertilizers. There are some ongoing efforts along this line from different stakeholders—government, scientific community, farmers, civil society, and industry. Legislation aimed at protecting the environment from nutrient run-off has been enacted by some governments, and policies based on this legislation are being implemented. For example, in compliance with the Federal Clean Water Act of 1972, some US states now require that agricultural site assessment indexes must include P source coefficients (Sharpley et al. 2003; Maynard and Hochmuth 2007) so that fertilizers, manures, and biosolids applied to agricultural soils can be evaluated on the basis of their potential to increase nutrient run-off.

The effort of the scientific community is the focus of this article. Research activities aimed at achieving better use efficiency of fertilizers, including the use of plant growth-promoting rhizobacteria (PGPR) and/or arbuscular mycorrhizae fungi (AMF) as supplements to fertilizers have steadily increased in the last two decades, as indicated from a search of a scientific literature database (Fig. 1). Historically, microbial inoculants have been used to achieve biological control or plant growth promotion. However, the impact of inoculants on nutrient uptake is a newer theme that has not yet been extensively investigated.

The premise of this review is that the goal of reducing fertilizers usage will be to this century what the goal of reducing pesticides was to the last century. The review discusses the diffuse nature of current reports in the literature concerning microbes as inputs towards a better use efficiency of fertilizers and the possibility of reducing the total amount of fertilizer usage. Some past studies reached conclusions that need to be critically discussed to avoid confusion among farmers, researchers, and policy makers. This review examines studies on different elements under various cropping systems where PGPR or AMF were used as inoculants. There is also a discussion on fertilization using manure and compost.

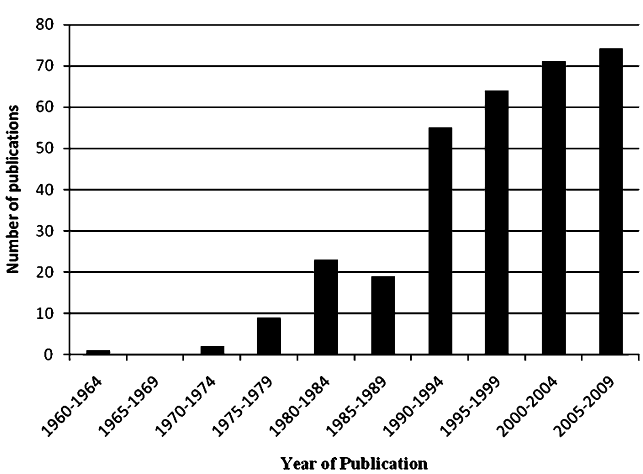

Fig. 1 Research activities measured by scientific publications from 1960 to 2009. This analysis was based on the ISI Web of Science database search in May 2009 using the keywords: "microorganisms and fertilizer use efficiency"

Fertilizer use in agriculture and the environmental impacts

Technological advances in agriculture are helping meet the food needs of an ever-increasing world population. Although the population has been growing and available land for agriculture has been shrinking, intensive agriculture that involves heavy and continuous use of fertilizers has ensured high crop productivity. As an example, increased use of fertilizers played an important role in the immense success in food productivity during the period of the green revolution (Tilman 1998). However, reports have shown that continuous use of fertilizers is generating environmental problems. Low efficiency in the uptake of fertilizer is a major factor that aggravates the negative environmental effects (Barlog and Grzebisz 2004). Over 50% of the applied N can be lost from agricultural systems as N_2, trace gases, or leached nitrate (Vitousek et al. 1997; Tilman 1998), and the impacts are usually long term and global in scope (Vitousek et al. 1997; Rabalais et al. 1998). Similarly, when P, another growth-limiting nutrient, is applied in high percentage, sometimes up to 90%, is precipitated by metal complexes in the soil (Rodriguez and Fraga 1999; Gyaneshwar et al. 2002) and can later lead to P pollution (Rodriguez and Fraga 1999; Sharpley et al. 2003).

Beside chemical fertilizers, amendments such as organic manure, compost, compost extract, and compost tea are also used in many parts of the world to enhance crop production and/or control plant pathogens. The changes in microbial activity of the soil based on the application of the organic materials are important, thus, some of the amendments are worthy of mention in this discussion. In sugarcane (*Saccharum* spp.), compost was shown to increase the uptake of some nutrients into the leaf; it was suggested that compost application to agricultural soil should provide better long-term fertility and lower off-site impacts, and it was recommended that conversion of municipal biosolids into compost for agricultural procution would be a desirable waste management strategy (Viator et al. 2002). Akanbi et al. (2007) showed that foliar spray of compost extracts from cassava (*Manihot esculenta*) peel and Mexican sunflower (*Tithonia rotundifolia*) help produce fluted pumpkin (*Telfairia occidentalis*) plants with comparable growth to those that received NPK fertilizer. In a different study with strawberry, Hargreaves et al. (2009) reported that compost tea enhanced the uptake of most macronutrients and micronutrients in strawberry plants in amounts that compared with municipal solid waste compost, ruminant compost, and inorganic mineral fertilizers. However, it is important to emphasize that agro-environmental problems are not limited to the use of chemical fertilizers but also occur with manures and compost (Mitchell and Tu 2006). Both animal waste and chemical fertilizers have the potential of environmental pollution (McLaughlin and Mineau 1995; Jarecki et al. 2008). Organic manures (fertilizers) contain N-rich materials, high extractable nutrients (P, K, calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn)), and can significantly raise soil fertility in the medium to long term (McLaughlin and Mineau 1995; Mitchell and Tu 2006). Mitchell and Tu (2006) noted that continued application of poultry waste will increase levels of soil nutrients, could cause a buildup of some nutrients, and loss of nutrients to the environment. Fertilizer, within the context of this paper, refers to both chemical fertilizers and manures unless specified otherwise.

Some of the environmental phenomena that have been linked to fertilizer use are briefly discussed hereafter. They include nitrate leaching, P run-off, groundwater pollution (Gyaneshwar et al. 2002; Sharpley et al. 2003), eutrophication of aquatic ecosystems and changes in the food web (Rabalais et al. 1998), reduction in biodiversity (McLaughlin and Mineau 1995), production of greenhouse gasses, global warming and acid rain, abnormal changes in soil pH, and changes in the salt concentration of soils (Mosier et al. 1996; Tilman 1998; Frink et al. 1999).

Nutrient leaching, run-off, and eutrophication of aquatic systems

Nitrate leaching and runoff in agriculture have been well documented and can lead to eutrophication and death of aquatic life (Turner and Rabalais 1995; Ottman and Pope 2000; Diaz and Rosenberg 2008). Ottman and Pope (2000) reported that leaching is inevitable; however, the severity of leaching can be controlled, in part, by the farmer since it is influenced by the type, rate, and timing of chemical fertilizer used. Some part of the applied fertilizer could run-off the farm to pollute other areas. Manures generated from livestock production have been reported to improve soil fertility but also lead to elevated concentration of P in run-off, thereby leading to eutrophication when P-rich soil particles erode from fields and reach surface waters (Ohno et al. 2005). Run-off of nutrients from fertilized farms across the Mississippi Basin increases nutrients in the Mississippi River and results in a “dead zone” in the Gulf of Mexico (Rabalais et al. 1998). The dead zone occurs when algal and vegetation growth increases in nutrient-enriched water with a resulting decrease in dissolved oxygen, fish kills, and death of many other aquatic life. Dead zones create severe stress on marine ecosystems and have been found in 400 locations worldwide covering an area more than 245,000 km^2 (Diaz and Rosenberg 2008).

Greenhouse gases, global warming, ozone layer depletion, and acid rain

Release of greenhouse gases (Flessa et al. 2002; Jarecki et al. 2008), ozone layer depletion (Ma et al. 2007), global warming, and acid rain are reported as negative impacts of fertilizer (Vitousek et al. 1997; Frink et al. 1999). Increases in emissions of CO_2, CH_4, and nitric oxide (N_2O), the three most important greenhouse gases, have been linked to fertilizer applications (Flessa et al. 2002). This increase could occur through gas fluxes from the soil surface or volatilization from plants (Mosier et al. 1996; Mulvaney et al. 1997). Increasing atmospheric N_2O is considered an important factor in ozone layer depletion (Ma et al. 2007). Gases such as N_2O and ammonia emissions from livestock and fertilizers contribute to acid rain and the acidification of soils and freshwater ecosystems (Norse 2003).

Plant–microbe interactions and impacts on plant growth, nutrient uptake, and yield

Microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Barea et al. 1998; Dobbelaere et al. 2001; Hodge et al. 2001; Bonfante 2003; Vessey 2003; Kloepper et al. 2004; Han and Lee 2005; Weller 2007; Adesemoye et al. 2008). Microbial inoculants include three major groups: (1) arbuscular mycorrhiza fungi (AMF), (2) PGPR, and (3) the nitrogen-fixing rhizobia, which are usually not considered as PGPR. Our focus in this review is on the first two groups. There is some discussion in the scientific literature on the role of specific strains of PGPR and AMF in plant growth promotion, N fixation, biofertilizer activities, or biological control of plant diseases (Koide 1991; Kloepper et al. 1999; Barea et al. 1998; Jetiyanon et al. 2003; Vessey 2003; Bashan et al. 2004; Morrissey et al. 2004), but there is need for more attention now especially in regards to nutrient interactions. Based on the beneficial effects of PGPR and AMF previously noted, studies using inoculant mixtures (Berg 2009) are very promising.

Benefits to plants from plant-PGPR interactions have been shown to include increases in seed germination rate, root growth, yield, leaf area, chlorophyll content, nutrient uptake, protein content, hydraulic activity, tolerance to abiotic stress, shoot and root weights, biocontrol, and delayed senescence (Mahaffee and Kloepper 1994; Raaijmakers et al. 1997; Bashan et al. 2004; Mantelin and Touraine 2004; Bakker et al. 2007; Yang et al. 2009). Other beneficial effects of PGPR strains include enhancing phosphorus availability

(Rodriguez and Fraga 1999); fixing atmospheric nitrogen (Bashan et al. 2004); sequestering iron for plants by production of siderophores (Raaijmakers et al. 1997; Bakker et al. 2007); enhancing biosynthesis of furanone flavor compounds in strawberry (*Fragaria* x *ananassa*; Zabetakis 1997); producing plant hormones (Gutierrez-Manero et al. 2001) such as gibberellins, cytokinins, and auxins; and synthesizing the enzyme 1-amino cyclopropane-1-carboxylate (ACC) deaminase, which lowers plant levels of ethylene, thereby reducing environmental stress on plants (Glick et al. 2007). The mechanisms behind plant-PGPR interactions are complex phenomena involving a combination of direct and indirect mechanisms, the details of which can be seen in the reviews by Glick et al. (2007) and Vessey (2003). One specific proposed mechanism by which PGPR affect nutrient uptake is by enhancing growth and development of plant roots, leading to root systems with larger surface area and increased number of root hairs, which are then able to access more nutrients (Biswas et al. 2000; Adesemoye et al. 2008).

The capacity of AMF to influence plant growth, water, and nutrient content has been widely reported over the years (Ames et al. 1983; Barea et al. 2002; Giovannetti et al. 2006). The AMF have a high-affinity P-uptake mechanism that enhances P nutrition in plants. The AMF are able to scavenge the available P through their hyphae that have large surface areas on which the extraradical hyphae act as a bridge between the soil and plant roots (Liu et al. 2000; Bianciotto and Bonfante 2002). The use of AMF also faces some problems. It is difficult to culture AMF in vitro, and the genetic basis of P solubilization and rhizosphere competence is not well understood (Amijee et al. 1989; Koide 1991). Also, a high concentration of the level of soil phosphate above 100 parts per million (ppm) could lead to a reduction in both hyphal growth and chlamydospore production by AMF (Amijee et al. 1989; Koide 1991), thus affecting P uptake and causing a reduction of the benefits to plants (Koide and Li 1990; Stewart et al. 2005). A reduction in hyphal growth could also affect N uptake as shown by Ames et al. (1983), who reported a correlation between mycorrhizal hyphal length and total N derived from applied ^{15}N-enriched ammonium sulfate $(^{15}NH_4)_2SO_4$, but did not observe any correlation in nonmycorhizal plants.

Considering the capacity of both PGPR and AMF to help plants in uptake of nutrients, a tripartite interaction of PGPR–plant–AMF is highly promising especially with the proposition that AMF may act as a vehicle to spread PGPR throughout the rhizosphere (Kim et al. 1998; Bianciotto and Bonfante 2002; Morrissey et al. 2004). It has been suggested that many natural AMF symbioses are tripartite associations involving AMF, the plant, and native bacteria (Bonfante 2003). In exploring the interactions between PGPR and AMF for better plant-use efficiency of inorganic fertilizers or manures, synergism is likely, but one must be cognizant that antagonism between PGPR and AMF is also a possibility. Many PGPR and AMF have been used separately and as combinations to investigate the impacts on the uptake of individual or multiple elements as discussed below. Also discussed in this paper are some studies conducted using molecular tools. Although the applications of the tools to sustainable agriculture are yet to be well understood, advances in genomic technology have provided substantial information in plant–PGPR and/or plant–AMF interactions.

Studies on nitrogen

The N cycle is an essential and complex biogeochemical cycle that has a great impact on soil fertility (Jetten 2008). The cycle is dominated by four major microbial processes: N fixation, nitrification, denitrification, and N mineralization (Ogunseitan 2005). Microbial inoculants have demonstrated significant roles in N cycling and plant utilization of fertilizer N in the plant–soil system (Ames et al. 1983; Briones et al. 2003; Adesemoye et al. 2009). Plant N uptake through symbiotic N fixation (Elsheikh and Elzidany 1997) and nonlegume biological fixation/nonassociative uptake have been reported widely in studies and many reviews have been written on the subject (Kennedy et al. 1997; Dobbelaere et al. 2001; Egamberdiyeva and Hoflich 2004; Vessey 2003; Bashan et al. 2004; Hernandez and Chailloux 2004; Wu et al. 2005; Shaharoona et al. 2008).

The summary of the studies previously listed are as follows. Wu et al. (2005) conducted a greenhouse study to evaluate the effects of four biofertilizers consisting of AMF (*Glomus mosseae* or *G. intraradices*) with or without N fixer (*Azotobacter chroococcum*), P solubilizer (*Bacillus megaterium*), and K solubilizer (*B. mucilaginous*) on the growth of maize (*Zea mays*). They reported that microbial inoculants increased the growth and nutritional assimilation (total N, P, and K) of maize and improved soil properties. In a pot experiment with soil collected from a nonfertilized field site near Tashkent, Uzbekistan, Egamberdiyeva, and Höflich (2004) demonstrated that inoculation with *Pseudomonas alcaligens* PsA15 and *Mycobacterium phlei* MbP18 led to increase in shoot and/or root N contents of cotton. Shaharoona et al. (2008) reported that pot and field trials with inoculation of *Pseudomonas fluorescens* (strain ACC_{50}) and *P. fluorescens* biotype F (strain ACC_{73}) showed increased use efficiency of N and P at all tested NPK fertilizer levels in wheat. The effect of ACC_{50} was higher in both pot and field tests than $ACC_{73,}$ with ACC_{50}causing 115%, 52%, 26%, and 27% increase over the noninoculated control at NPK application rates of 25%, 50%, 75%, and 100% of recommended doses, respectively.

Furthermore, Amir et al. (2005) reported enhanced uptake of N and P in oil palm seedlings in Malaysia, following PGPR inoculation in the field nursery. Aseri et al. (2008) conducted experiments in the field in India and assessed the effectiveness of PGPR (*Azotobacter chroococcum* and *A. brasilence*) and AMF (*Glomus mosseae* and *G. fasciculatum*) on the growth, nutrient uptake, and biomass production of pomegranate (*Punica granatum* L.). Strains were applied individually or in combinations. Results showed that dual inoculation of PGPR and AMF led to higher biomass production and increase in the uptake of N as well as P, K, Ca, and Mg in pomegranate seedling. Increase in N and P uptake was suggested to result from improved symbiotic N_2 fixation and improved phosphatase activity. The study by Adesemoye et al. (2008) confirmed that inoculation with mixed strains were more consistent than single strain inoculations.

Nitrogen fixation has been proposed as a mechanism involved in enhanced N uptake of inoculated plants. A specific example is *Azospirillum* spp. enhanced plant N uptake and plant growth promotion in which nitrogen fixation was the first reported mechanism as reviewed by Dobbelaere et al. (2001) and Bashan et al. (2004). It must be emphasized that nitration fixation is not the only mechanism; other mechanisms that have been proposed in *Azospirillum* include production of phytohormones leading to improved root growth, water adsorption, and mineral uptake (e.g., phosphate solubilization), proton, and organic acid extrusion. It is well reported that uptake of N, P, K, and micronutrients are significantly enhanced in plants inoculated with *Azospirillum* in both the greenhouse and field. It is crucial to point out that successful plant root colonization is very important in *Azospirillum* and other PGPR in achieving enhanced nutrient uptake. Details on *Azospirillum* can be found in Dobbelaere et al. (2001) and Bashan et al. (2004).

In similar ways, the possibility of AMF and other PGPR to fix N are being examined, and molecular tools have been helpful in this effort (Minerdi et al. 2001). Putative nitrogenase coding genes (*nif* operon), in a 30 kb DNA region, have been described in bacteria, and the transcriptional organization has been studied (de Zamaroczy et al. 1989; Galimand et al. 1989). Nitrogenase, the enzyme responsible for N fixation, has two components: I (an $\alpha_2\beta_2$ tetramer encoded by *nifD* and *nifK* genes) and II (a homodimer encoded by *nifH* gene). These two components are conserved in structure, function, and amino acid sequence through diazotorphs. The genes are commonly reported to regulate lateral root development and long distance movement of nitrogen (de Zamaroczy et al. 1989; Ueda et al. 1995; Minerdi et al. 2001). Minerdi et al. (2001) examined *Burkholderia* spp. for the presence of the N-fixation gene and its expression in plants using the genomic library constructed for *Glomus margarita* spores (BEG 34; a symbiont), which also contained the bacterial genome. Minerdi et al. (2001) were able to describe the *nif* operon. They reported that *Burkholderia* NifH, NifD, and NifK proteins have high sequence similarity to those of *Azospirillum brasilense*. The expression of *nif* genes indicates a potential to fix nitrogen (Minerdi et al. 2001).

The nitrogenase enzyme complex has been credited for the capacity of some PGPR to convert nitrogen into ammonia in a free state (Egener et al. 1999). Some nitrogen-fixing Gram-negative bacteria have been identified as endophytes of gramineous plants, e.g., *Azocarcus* sp. in Kalla grass, rice, and wheat. Egener et al. (1999) studied root-associated GUS (histochemical β-glucuronidase) and *nifH* expression with the objective of monitoring the establishment of nitrogen-fixing bacteria (*Azoarcus* sp.) on or in rice roots. The authors observed that a primary step in assessing the metabolic capacities of beneficial bacteria in associations with the host plants is to localize the expression of bacterial genes of interest in the host plant. Egener et al. (1999) noted that the presence of combined nitrogen such as ammonia has a strong impact on the expression of *nif* gene in most diazotrophs. Also, Vande Broek et al. (1993) estimated associative *nifH* expression both qualitatively and quantitatively in *A. brasilense* on wheat roots through gusA fusion plasmid system. However, as noted by Mantelin and Touraine (2004), there is no clear evidence that the expression of *nif* genes or active N_2 fixation by PGPR will translate into measurable transfer of the fixed N to the plant. Understanding of the key factors governing microbial ecology of the rhizosphere is highly needed (Hardy and Eaglesham 1995) but has yet to be fully achieved. Nonetheless, we share in the conclusion of Bhattacharjee et al. (2008) that with progressive understanding of the interactions between nitrogen-fixing bacteria and cereal crops, the world is closer to the dream of developing an ecofriendly nutrient source for cereal crops.

Studies on phosphorus

Phosphorus is another growth-limiting nutrient. The biggest reserves of P are rock phosphates, which are highly insoluble. Although most agricultural soils have large amounts of inorganic and organic P, these are immobilized and mostly unavailable. Hence, only a very low concentration of P is available to plants, and many soils are actually P deficient. One major reason that P is not readily available to plants is because of the high reactivity of P with some metal complexes such as iron (Fe), Al, and Ca leading to the precipitation or adsorption of between 75–90% of P in the soil (Igual et al. 2001; Gyaneshwar et al. 2002). While plant available N is present in millimolar amounts, plant-available P is usually in micromolar. Even when P fertilizers are added to soils, they may not be absorbed by plants because P can easily get bound in soil or becomes sparingly soluble, and so, less than sufficient amount of P would be available for crop growth and yield (Gyaneshwar et al. 2002). The farmer may then have to add large amount of fertilizers (Ohno et al. 2005), and a significant part of the P will later constitute an environmental problem.

Inoculants, PGPR and AMF, are playing significant roles in the solubilization of inorganic phosphate and mineralization of organic phosphates (Mahmood et al. 2001; Tawaraya et al. 2006). There is evidence relating to inorganic phosphate (P_i) transporter and its expression in the external hyphae of AMF, which is important in the uptake of P_i and transfer from the AMF to plants (Harrison and van Buuren 1995). In one study, 36 bacterial strains, with the capacity to solubilize mineral phosphate, were characterized from Taiwan after screening them with tricalcium phosphate medium. The principal mechanism for their solubilization capacity was reported as production of organic acids (Chen et al. 2006). Some studies have corroborated this by cloning two genes (*PQQ* synthase and *gabY*) that are involved in gluconic acid production as reviewed by Igual et al. (2001). Gluconic acid is the principal organic acid produced by many organisms, but other acids include 2-ketogluconic, acetic, citric, glycolic, isovaleric, isobutyric, lactic, malonic, oxalic, propionic, and succinic acids (Rodriguez and Fraga 1999; Chen et al. 2006).

Organic P usually accounts for 30% to 65% of total P in soils and must be converted to inorganic or low-molecular weight organic acids before they can be assimilated by plants. Although the structure of the different forms of organic P in soils is not well understood, the common forms are inositol phosphatases, phosphoesters, phosphodiesters (phospholipids and nucleic acids), and phosphotriesters. A large part of the organic P is inositol phosphatases (phytate), accounting for half or more of organic P in soils and are the most important in plant nutrition (Rodriguez and Fraga 1999; Zimmermann 2003). Phosphatases refer to any enzyme that can hydrolyze phosphate esters and anhydrides. These include phosphoprotein phosphatases, phosphodiesterases, diadenosine tetraphosphatases, exonucleases, 5'-nucleotidases, phytases, alkaline and acid phosphatases, phosphomonoesterases, etc (Zimmermann 2003). Phosphatases are sometimes described as phosphomonoesterase in the literature. The possibility of phosphatases to be mobilized for plant available P from soil organic sources by AMF and the secretion of phosphatases through some mycorhizal fungi has been reported (Antibus et al. 1992). These have also been shown in some PGPR, including genus *Bacillus* (Idriss et al. 2002), *Pseudomonas*, and *Rhizobium*, as reviewed by Rodriguez and Fraga (1999).

Molecular biology tools have been used to elucidate plant-microbe interactions in P metabolism (Rodriguez et al. 2000; Chen et al. 2006). Minder et al. (1998) indicated that the genetic control system of phosphate (PO_4) uptake is based on the PO_4 regulatory protein PhoB, which is mediated by the transmembrane sensor protein PhoR. They suggested that phosphorylated PhoB acts as a transcriptional activator to the *pho* box in the promoter region of genes belonging to the *pho* regulon. They explained that the product of the *phoB* gene regulates the cellular response to environmental phosphate limitation. Although *Bradyrhizobium japonicum* is an N fixer, after the study with *B. japonicum* on soybean, it was concluded that *phoB* was required for phosphate-limited growth but not for symbiotic N fixation (Minder et al. 1998).

In addition, two phosphate transport systems—a low-affinity phosphate inorganic transport system and a high-affinity phosphate-specific transport system (transporter operon, *pst*)—in bacteria were previously described (Ruiz-Lozano and Bonfante 1999). Subsequently, Ruiz-Lozano and Bonfante (1999) investigated the role of *Burkholderia* sp. in AMF P metabolism and its possible shunting off in P transfer from fungus to the plant. *Burkholderia* is an intracellular bacteria present throughout the life cycle of many AMF species of *Gigasporaceae*. These authors cloned and characterized an operon for a Pst-like system. The open reading frames in the operon and the protein they encode (PstA, PstB, PstC, PstS, and PhoU) were studied. The conclusion was that *Burkholderia* contains a genomic region similar to the *pst* operon of *E. coli* in sequence, order, and number of genes and has the potential to take up P from the environment and affect P uptake by the AMF host (*Glomus margarita*) and the plant symbiont. With the possession of a DNA region having the nitrogenase-coding genes (*nif* operon), the intracellular *Burkholderia* can also affect N uptake. These types of approaches are promising towards better understanding the role of the interaction of bacteria and AMF in plant nutrient uptake.

Studies on other elements

Inoculants have been shown to influence the uptake of many other elements in addition to N and P (Peix et al. 2001; Khan 2005; Wu et al. 2005; Adesemoye et al. 2008). In a review, Khan (2005) observed that inoculation with many PGPR such as *Pseudomonas* and *Acinetobacter* strains had resulted in enhanced uptake of Fe, Zn, Mg, Ca, K, and P by crop plants. In a study with strains of *Mesorhizobium mediterraneum* inoculated onto chickpea and barley, K, Ca, and Mg in addition to P and N contents significantly increased in both plants (Peix et al. 2001). Kohler et al. (2008) also demonstrated the effects of PGPR (*Pseudomonas mendocina* Palleroni) and AMF (*G. intraradices* and *G. mosseae*) on uptake of N, P, Fe, Ca, and manganese (Mn) in lettuce (*Lactuca sativa* L. cv. Tafalla) under three different levels of water stress in Spain. Han and Lee (2005) reported an increased uptake of P and K when soil was fertilized with rock P and K and co-inoculated with P solubilizing bacteria *B. megaterium* and K solubilizing bacteria *B. mucilaginosus.* Sheng and He (2006) reported improved uptake of K through the inoculation of PGPR *B. edaphicus* strains NBT and suggested that the production of organic acids (citric, oxalic, tartaric, succinic, and α-ketogluconic) by the strain and its mutants lead to chelation of metals and mobilization of K from K-containing minerals.

Giri and Mukerji (2004) reported significant increase in Mg concentrations in seedling tissues of *Sesbania aegyptiaca* and *S. grandiflora* after application of AMF *Glomus macrocarpum*, compared with nonmycorrhizal seedlings in saline soil. However, this was different from the results of Azcon-Aguilar et al. (1986) who suggested that AMF are not involved in Mg nutrition since they observed a lower concentration of Mg in shoot of soybean inoculated with AMF. Liu et al. (2000) reported an increase in acquisition of Fe, Zn, Cu, and Mn by mycorrhizal maize. Sulfur (S) and Fe uptake have been achieved through sulfur-oxidizing bacterial inoculant and siderophore-producing bacteria, respectively (Banerjee et al. 2006; Bakker et al. 2007). Biswas et al. (2000) reported a significant increase in Fe uptake in lowland rice through inoculation of *Rhizobium leguminosarum* bv. *trifolii* E11. They suggested that the increased uptake of Fe, P, and K was associated with higher N rates but higher N was a result of mechanisms other than biological N fixation.

Isotope-labeling techniques

Isotope-labeling techniques are being used to study the impacts of both PGPR and AMF on nutrient uptake, especially P and N (Nayak et al. 1986; Hodge et al. 2001; Tu et al. 2006; Barea et al. 2007; Adesemoye et al., unpublished). Zapata and Axmann (1995) observed that one adequate approach for assessing the availability of P in rock-phosphate materials to crops is through the use of $^{32}P/^{33}P$ isotope tracers. When isotopic P-labeled soil is used, estimation of the sources of P in plant tissues is easily estimated based on the specific activity in the plants. By amending a neutral-calcareous soil with ^{32}P-^{45}Ca-tricalcium phosphate, Azcon-Aguilar et al. (1986) were able to estimate the effect of AMF (*Glomus mosseae* and *Glomus* spp.) and two phosphate solubilizing bacteria on the growth and nutrition of soybean. Barea et al. (2002) were able to evaluate the interactive effects of P-solubilizing rhizobacteria, AMF, and *Rhizobium* in legumes using the isotopes ^{32}P and ^{15}N.

The stable isotope ^{15}N labeling has been used relatively more with *Azospirillum* than other PGPR species (Nayak et al. 1986; Belimov et al. 1995). Nayak et al. (1986) used the technique of ^{15}N to monitor the inoculation effect of *A. lipoferum* on N uptake in rice. Recently, Adesemoye et al. (unpublished) inoculated a mixture of two PGPR strains (*B. amyloliquefaciens* IN937a and *B. pumilus* T4) onto tomato in a greenhouse study and evaluated the effect of the PGPR on plant uptake of applied fertilizer N using different rates of ^{15}N-depleted ammonium sulfate. The use of ^{15}N isotope and its basis in monitoring the movement of N in biological N fixation, mineralization-immobilization of N, plant recovery of applied N, and movement of N (including enriched and depleted ^{15}N) were detailed in Hauck and Bremner (1976). Isotope techniques are proving very useful in understanding the inoculants-enhanced plant nutrient-uptake paradigm, but the technique in itself is not a guarantee for data reliability. The experimental design, collection and analysis of data, and the capability of the researcher in ^{15}N data interpretation are very important.

Issues arising from recent studies

As the information on the effects of inoculants on nutrient uptake keeps increasing, there is a need for a continuous discussion of emerging scientific data and reevaluation of methodologies. This will help towards achieving the overall goal and ensure that scientific information is not confusing to farmers and researchers alike, especially those new to the field. This would lead us into the discussion of the following published works. Egamberdiyeva (2007) used two soil samples (a nutrient-poor calcisol soil from Uzbekistan and a nutrient-rich loamy sand from Germany) to study the impact of PGPR on nutrient uptake in maize in pot experiments. This author indicated that bacterial inoculation had better stimulating effect on the growth and nutrient (N, P, and K) uptake of maize in nutrient-deficient calcisol soil than loamy sand, which was contrary to the common assumption that the usefulness of PGPR is limited under nutrient deficient conditions (Khan 2005). From the design, it was not indicated that fertilizer was applied to any of the treatments for the whole duration of 4 weeks that maize growth lasted. Information about fertilizer application would have been helpful in comparing the effects of the inoculants.

Canbolat et al. (2006) provided a good basis for comparison of the impact of inoculants with fertilizer. The study was conducted with barley seedlings in a design of eight treatments, three soil compaction, and three harvest times in a pot experiment. The eight treatments included (1) control (without bacteria or fertilizer addition), (2) N fertilizer (40 mg N kg^{-1} soil), (3) P fertilizer (20 mg P kg^{-1} soil), (4) NP fertilizer (40 mg N kg^{-1} soil+20 mg P kg^{-1} soil), (5) *Bacillus* RC01, (6) *Bacillus* RC02, (7) *Bacillus* RC03, and (8) *Bacillus* M-13. It was shown that available P and N were significantly greater in the first harvest at 15 days after planting (DAP) compared with 30 and 45 DAP, which indicated that the impact of inoculants on nutrient uptake could depend on time or the stage of growth of the plant. Similarly, Adesemoye et al. (2009) observed that time of sampling, i.e., the plant's stage of growth, significantly impacted on the effectiveness of the inoculants. Furthermore, Canbolat et al. (2006) reported increases in N and P content of plant dry matter with each inoculated *Bacillus* strain compared with the control. It was also shown that the amounts of N and P in plants inoculated with *Bacillus* were lower than the plants that were fertilized with N, P, or NP fertilizers. This is an indication that inoculants were not able to fully replace fertilizer, though it would have been more informative if Canbolat et al. (2006) had compared joint applications of fertilizer and inoculants with separate applications of each.

The study by Elcoka et al. (2008) was somewhat similar to Canbolat et al. (2006) in terms of design. Elcoka et al. (2008) studied chickpea inoculated with strains of *Rhizobium*, N_2-fixing *Bacillus subtilis* OSU-142, and P-solubilizing *B. megaterium* M-3 in comparison with mineral fertilizer application and a noninoculated, non-fertilized control in "controlled environments" and in the field. The design of the experiments is interesting, and it gives room for comparison of inoculants and fertilizer. The authors showed that single, double, and triple inoculations significantly increased all parameters measured (including N content), with equal or higher proportion compared to treatments with N, P, and NP fertilizers in controlled experiments. In the field trial, the trend was similar for pod number and seed yield. However, the conclusion of Elcoka et al. (2008) that double and triple combinations of inoculants may substitute for NP fertilizers in chickpea production is a point of concern. Contrary to this, Shaharoona et al. (2008) showed that the effectiveness of their PGPR strains (*P. fluorescens* [ACC_{50}] and *P. fluorescens* biotype F [ACC_{73}]) were fertilizer-dependent. We have not seen enough data for us to concur with Elcoka et al. (2008) that inoculants will replace fertilizer; rather many studies, for example Adesemoye et al. (2009), have shown that microbial inoculants are good and reliable supplements to fertilizer.

Model for inoculants-enhanced plant nutrient use efficiency

One current proposition towards solving agro-environmental problems is integrated nutrient management (INM), which does not aim to remove fertilizer totally in the short run but to reduce the negative impacts of overuse of fertilizers containing N, P, and other elements. The INM system promotes low chemical input but improved nutrient-use efficiency by combining natural and manmade sources of plant nutrients in an efficient and environmentally prudent manner. This will not sacrifice high crop productivity in the short term nor endanger sustainability in the long term (Gruhn et al. 2000; Adesemoye et al. 2008). In a recent 3-year field study, it was demonstrated that PGPR-elicited plant growth promotion resulted in enhanced N uptake by plant roots (Adesemoye et al. 2008). It was concluded in the paper that the increase in plant N content might have resulted from increased fertilizer N utilization efficiency in an INM system. These current approaches in microbe-plant-fertilizer interactions could be explained using the model below (Fig. 2).

In Fig. 2a, the amount of fertilizer applied to plants is usually large; in Fig. 2b, the part of the applied fertilizer taken up by plants is usually small, ranging between 10% to 40% depending on soil type, fertilizer type, and plant; and in Fig. 2c, the part of the applied fertilizer that is lost could be in the range of 60% to 90% of the original amount of fertilizer or manure applied (Hardy and Eaglesham 1995; Rowarth 1997; Hood et al. 1999; Gyneshwar et al. 2002; Barlog and Grzebisz 2004; Kleinman et al. 2005). As have been previously discussed, examples of the route of nutrient loss include N leaching, P fixation, and nutrient run-off among others. Then, the question being asked is whether it is possible to reverse the trend of (1) loosing high

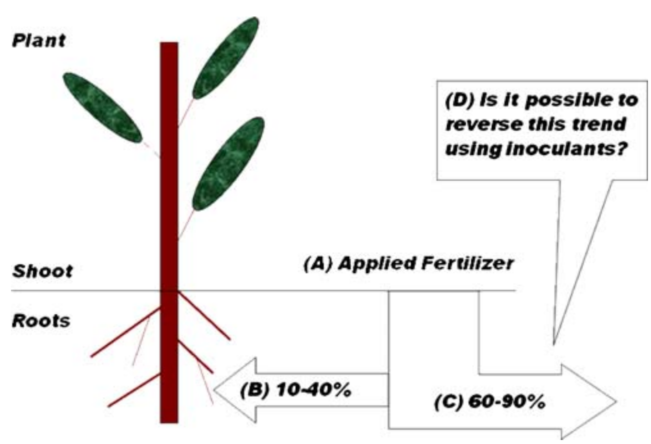

Fig. 2 Model for improved plant nutrient use efficiency with inoculants. **a** Total amount of fertilizer or manure applied to plants, **b** 10% to 40% of the applied fertilizer or manure is taken up by plants, and **c** 60% to 90% of the applied fertilizer or manure is lost

percentage of applied fertilizer and (2) applying large amounts of fertilizers by supplementing reduced fertilizer with inoculants while maintaining plant growth and high yield comparative to the use of full recommended fertilizer rates?

Conclusion

Obviously, the use of chemical fertilizers and manures cannot be eliminated at this time without drastically decreasing food production. At the same time, the harmful environmental side-effects of fertilizer use, such as the expanding dead zones in marine systems worldwide, cannot go unabated. Hence, there is an urgent need for integrated nutrient management that targets agricultural inputs and lowers the adverse environmental impacts of agricultural fertilizers and practices. Better understanding of the interactions between microbe, fertilizer, and plants is very important. There is need for more information along the models previously discussed (Fig. 2), the application of which is two-pronged. First, by getting more of the applied nutrient into the plant tissues through the help of microbial inoculants, fewer nutrients are lost to the environment after the season. The possibility of nutrient run-off or leaching is further reduced if the crop residues are removed from the field. Second, it will become possible to apply lower amounts of fertilizers after achieving increases in the use efficiency of the applied fertilizers. In each case, reduction in agro-environmental pollution will be achieved. Results have shown that joint inoculation of strains of PGPR and/or AMF or commercial formulations containing multiple strains has been able to circumvent earlier reported inconsistencies. Therefore, the application of this model will be better with a design that incorporates multiple strains. Meanwhile, some specific areas need to be better studied.

One aspect that remains to be convincingly proven in the literature is the fate of nutrients solubilized in the soil by inoculants. As a specific example, the correlation between solubilization by microorganisms and practical uptake of the solubilized P by plant is not yet clear. Studies using liquid or solid media under controlled environments have shown that microorganisms are able to solubilize P from insoluble sources (Peix et al. 2001; Idriss et al. 2002; Ivanova et al. 2006). However, data on what proportion of the laboratory-based P solubilization is taken up by plants in the field or used by the microorganism for its development are not well defined in the literature. These and related information will help in determining the level of insoluble phosphorus and inoculants that would be needed for practical purposes in the field. This is important because the amount of P solubilized, P need of the bacteria, root exudation of the specific plant, and soil conditions (including soil P status, P sorption capacity, and pH) are among many possible factors that could affect whether the P that is solubilized is taken up by plants or not. Further studies with focus on similar issues with other elements and the molecular mechanisms of the impacts of microbes on plant nutrition and fertility management will help improve our understanding of how to use microbial inoculants to decrease harmful effects of fertilizers.

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