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RESEARCH ARTICLE

Up to 52 % N fertilizer replaced by biofertilizer in lowland rice via farmer participatory research

Michael T. Rose · **To Lan Phuong** · **Dang Kieu Nhan** ·
Phan Thi Cong · **Nguyen Thanh Hien** · **Ivan R. Kennedy**

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Abstract Rice production needs to rise substantially without increasing inputs such as chemical fertilizers to feed the world's growing population in a sustainable manner. In this regard, plant growth-promoting microorganisms, formulated as inoculant biofertilizers, show strong potential by improving nutrient use efficiency. However, the practical use of biofertilizers by farmers remains limited because of inconsistent results under field conditions. We hypothesized that biofertilizer performance depends on the amount and type of chemical fertilizer applied in concert with the biofertilizer and that such knowledge can improve inoculation efficacy. Farmer participatory field experiments were conducted at 20 different farms from two localities in the Vietnamese Mekong Delta over four growing seasons. On each farm, one half of a split-plot was treated with chemical fertilizer at conventional rates. The remaining area was given only 50–80 % of the usual chemical fertilizer rates but supplemented with the commercial biofertilizer BioGro containing four plant growth-promoting microorganisms. Our results demonstrate that the biofertilizer can replace between 23 and 52 % of nitrogen (N) fertilizer without loss of yield but cannot substitute for phosphorus (P) fertilizer. In addition, we found that up to 45 % of the variability in biofertilizer performance is related to the amount and timing of N, P, and K fertilizers applied to the crop. Importantly, the yield response to both biofertilizer and N fertilizer is strongly affected by the seasonal growing conditions. Overall, our findings show for the first time that farmer participatory experiments can be used to increase the efficacy of biofertilizers through manipulating chemical fertilizer inputs. This new information will accelerate the uptake of biofertilizer technology if managed correctly.

Keywords Biofertilizer · Sustainable agriculture · Rice · Fertilizer · Nutrient use efficiency · Plant growth-promoting microorganism · Nitrogen

1 Introduction

Rice cultivation is essential for world food security but relies on chemical fertilizer inputs to sustain high yields (Dobermann and Fairhurst 2000). In the Vietnamese Mekong Delta, nitrogen (N), phosphorus (P), and potassium (K) fertilizers usually represent 30–35 % of input costs, and these costs have been steadily rising over the past decade, while at the same time, many state subsidies are being reduced. Unfortunately, the efficiency of chemical fertilizer use in rice systems in Southeast Asia is generally low, with less than 50 % of the applied chemical fertilizer actually being used by the growing crop (Choudhury and Kennedy 2005). Not only is this inefficiency an economic burden, but the unused fertilizer portion can be lost through surface water

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M. T. Rose (✉)
School of Chemistry and School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia
e-mail: mick.rose@monash.edu

T. L. Phuong · D. K. Nhan
Mekong Delta Development Research Institute, Can Tho University, Can Tho, Vietnam

P. T. Cong
Agriculture Green Future, 175/2 Nguyen Thien Thuat, District 3, Ho Chi Minh, Vietnam

N. T. Hien
Biofertilizer Action Research Center, 12 Hang Chuoi, Hanoi, Vietnam

I. R. Kennedy
Faculty of Agriculture and Environment, The University of Sydney, Eveleigh, NSW 2015, Australia

runoff or leaching to result in the eutrophication of ecosystems or contamination of ground water. Furthermore, overuse of N fertilizers significantly contributes to global greenhouse gas emissions. This occurs throughout the lifecycle of N fertilizers and includes high CO_2 emissions during production and transport, as well as nitrous oxide (N_2O) emissions following application in the field (Choudhury and Kennedy 2005).

Because of the heterogeneity of soils and seasonal climatic differences, the yield response to fertilizer applications can be inconsistent throughout the year at regional and even field scales (Tan et al. 2004). Farmers therefore need to optimize fertilizer applications in line with their specific environment and management practices in order to maintain high yields without sacrificing profit (Peng et al. 2010). Site-specific nutrient management requires both theoretical and practical knowledge of nutrient dynamics and budgets for the agricultural system in question and necessitates strong involvements from the landholder/farmer for successful implementation.

A promising strategy for improving nutrient use efficiency is the application of plant growth-promoting rhizobacteria (Vessey 2003; Adesemoye et al. 2009). Worldwide studies have shown that certain microorganisms associated with plant roots can improve plant nutrition, growth, and yield. Improved nutrition can be facilitated directly through microbial processes such as atmospheric N fixation or mobilization of indigenous soil nutrients or indirectly through microbial stimulation of plant root systems to increase the acquisition of indigenous or fertilizer-applied nutrients (Richardson et al. 2009; Vessey 2003). A number of different mechanisms can be involved in indirect root stimulation, including phytohormone production, pathogen suppression and protection from stress conditions such as salinity and drought through polysaccharide production (Avis et al. 2008; Yang et al. 2009). It is possible that even small improvements to plant growth through either direct or indirect mechanisms can lead to positive feedbacks through better soil exploration, thereby increasing nutrient and water use efficiencies and reducing the need for chemical fertilizers.

Despite these prospects, there is no guarantee that plant growth-promoting microorganisms isolated via phenotypic tests will improve growth in soil-cultured plants under glasshouse conditions (Smyth et al. 2011). Repeated trials under controlled environmental conditions in the glasshouse or nursery are recommended for beneficial microorganisms to ensure consistent growth promotion compared to uninoculated controls (Deaker et al. 2011). Even then, the successful transfer of recognized growth-promoting microbes from glasshouse to field conditions is difficult (Lucy et al. 2004), with zero or even negative effects often observed depending on site conditions, choice of cultivar, and management practices (Sasaki et al. 2010; Yanni and Dazzo 2010). Because of the inconsistent performance of plant growth-promoting microorganisms under field conditions perhaps because of inadequate quality control combined with a lack of understanding of the ecology and survival of these microorganisms, practical application of inoculant biofertilizers remains limited (Martinez-Viveros et al. 2010). Dual management of chemical fertilizers together with biofertilizers requires an additional level of knowledge for farmers and agronomists in order to make successful site-specific recommendations.

Recently, we have investigated the efficacy of a multistrain inoculant biofertilizer for improving rice yields under full and reduced chemical fertilizer regimes in Vietnam (Cong et al. 2011; Kennedy et al. 2008; Nguyen et al. 2003; Phan et al. 2009). Amongst our observations, the potential for the inoculant biofertilizer to supplement chemical N fertilizer application stands out as one of the more consistent traits in both glasshouse and field experiments, but strong seasonal influences are evident (Phan et al. 2009). Similar interactions between growth-promoting rhizobacteria and N fertilizers have been observed elsewhere, but as far as we are aware, the field optimization of inoculant biofertilizers in conjunction with chemical fertilizers has not been directly attempted or at least reported. If the application and use of biofertilizers for increasing yields while reducing chemical fertilizers is to become reliable and widespread, better practical information on how to ensure biofertilizer efficacy is needed, especially under realistic field conditions.

Our objectives were to determine the effects of a commercial inoculant biofertilizer on chemical fertilizer reductions and rice yields in terms of magnitude and reproducibility over multiple growing seasons. We hypothesized that the efficacy of the biofertilizer is dependent, in part, on the rate and timing of supplementary chemical fertilizer application. In testing this hypothesis, we deliberately encouraged participating farmers to modify the amount of chemical fertilizer they applied in conjunction with the biofertilizer according to their own preference (Fig. 1). This experimental design produced a

Fig. 1 Rice plots treated with biofertilizer also received reduced chemical fertilizer as compared with control plots

range of fertilizer management strategies under which the biofertilizer performance could be assessed, while taking advantage of farmer observation and experience to accelerate optimization.

2 Materials and methods

2.1 Study area and experimental design

Field experiments were conducted on 20 rice cropping farms in the Mekong Delta, Vietnam. Ten farms were selected from each of two localities: Cai Lay District and Phung Hiep District. All farms had a history of high-yielding rice production for at least 20 years prior to these field experiments. In this area of the Mekong Delta, three crops of rice are regularly grown per year, including a dry season crop planted after the annual flood in September, an early wet season crop, and a late wet season crop. The timing of these crop cycles is given in Table 1. Field experiments were conducted in four successive seasons from summer 2009 to winter 2011, commencing with a late wet season crop prior to the flood.

Prior to the first crop, the soils at each farm were classified and analyzed for EC, pH, extractable mineral nitrogen ($NO_3^-$and NH_4^+), total nitrogen, and Olsen extractable phosphorus. The soils at all farms were classified as Umbri Plinthic Gleysols (alluvial and well-drained soils with mottling in 30–60 cm horizon). Soil chemical characteristics at each farm are given in Supplementary file 1.

The general experimental design consisted of paired plots at each of the farms. For each farm, a plot of 2,000 m^2 was devoted to the experiment. The plot was equally split into two subplots, one for biofertilizer treatment and another one for farmer's normal practice as the control. The subplots were separated with small bunds to ensure isolation from each other and no mobility of water and applied fertilizers. One plot received *conventional* farmer fertilizer application and the other plot received a biofertilizer application at a rate of 100 kg ha^{-1} combined with a reduction in chemical fertilizer rate. Fertilizer application rates in the control plots and the percent reduction in the biofertilizer plots differed at each farm depending on individual farmer practice. A summary of fertilizer rates is given in Table 1.

2.2 Biofertilizer

The biofertilizer used in the field experiments is a commercial product known as "BioGro," containing four different microbial strains in a peat carrier material. The strains have been previously identified as *Pseudomonas fluorescens* (Pf1N), *Bacillus subtilis* (BsB9), *Bacillus amyloliquefaciens* (BaE19), and *Candida tropicalis* (CtHY) (Kennedy et al. 2008). The biofertilizer was produced from pure culture stock held at the Biofertilizer Action Research Center in Hanoi or the Institute of Agricultural Sciences, Ho Chi Minh City, Vietnam, by growing broth cultures for 72–96 h (Nguyen et al. 2003). Broths were then mixed into peat that had been neutralized with $CaCO_3$ to a pH of 7. Quality control was performed to ensure that each batch of biofertilizer contained a minimum of 10^6 colony-forming units of each strain per gram biofertilizer by conducting selective plate counts prior to the dispatch of product to farms according to the methods of Rose et al. (2011). Considering an application rate of 100 kg biofertilizer ha^{-1}, the minimum number of each strain applied was therefore 10^{11} cfu ha^{-1}. BioGro inoculant was applied at sowing by broadcasting by hand.

2.3 Rice agronomy and harvest

Rice varieties were high-yielding varieties with duration of 90–100 days but differed amongst localities and crop seasons. In Cai Lay, farmers generally sowed OM6162, while in Phung Hiep, the main varieties used were OM4218 and IR50404. Seed was sown by hand at rates of 120 kg/ha in Cai Lay and 150 kg/ha in Phung Hiep. In control (farmer practice) plots, chemical fertilizer was applied in three to four splits for total rates of 70–100 kg N ha^{-1}, 50–80 kg P_2O_5 ha^{-1}, and 50–70 kg K_2O ha^{-1} in Cai Lay and four splits for total rates of 80–120 kg N ha^{-1}, 40–70 kg P_2O_5, and 50–70 kg K_2O ha^{-1} in Phung Hiep. *Early* applications were designated as fertilizer applied from 0–30 days after sowing; *late* applications were designated as fertilizer applied after 30 days after sowing. In plots treated with biofertilizer, chemical fertilizer was applied at the same time as control plots but at reduced rates as outlined in Table 1. Plots were continuously flooded with water levels held at 50–100 mm above the soil surface. Weeds, diseases, and insects were controlled by integrated pest management using pesticides only when necessary. No noticeable crop damage from pests was observed throughout the experiment. The grain yield of rice was determined by harvesting three sample areas of 5 m^2 from each plot. Grain moisture was measured and yield was converted into yield per hectare at standard moisture of 14 %.

2.4 Statistical analyses

The complete data set was analyzed using a general linear model in which locality by season factors were reclassified as environments (Raman et al. 2011), resulting in eight different environments (two localities × four cropping seasons). The initial model included biofertilizer agronomy, environment, and their interaction as fixed factors, with individual farms nested within environments as a block effect (model 1). Because the amount of chemical fertilizer added in combination with the biofertilizer treatments varied depending on farm and season, model 1 assessed the effect of the biofertilizer

Table 1 Cropping seasons and chemical fertilizer applications for Cai Lay and Phung Hiep trial sites. Mean chemical fertilizer applications (kilograms per hectare) are given for conventional farmer practice (FP) and the biofertilizer system (BF) together with the percentage reduction (%R) in chemical fertilizer under the BF as compared to FP

Cropping season		Late wet season 2009		Dry season 2010		Early wet season 2010		Late wet season 2010	
Locality		Cai Lay	Phung Hiep	Cai Lay	Phung Hiep	Cai Lay	Phung Hiep	Cai Lay	Phung Hiep
Sowing date		15 May 2009	5 July 2009	5 December 2009	11 December 2009	15 March 2010	26 March 2010	22 June 2010	24 June 2010
Harvest date		16 August 2009	13 October 2009	10 March 2010	16 March 2010	23 June 2010	4 July 2010	25 September 2010	2 October 2010
Growth duration (days)		93	100	95	95	100	100	95	100
Average temperature (°C)		27.6	27.1	26.1	26.7	28.6	29.2	27.1	27.4
Total rainfall (mm)		549	570	39	16	401	222	824	554
Solar radiation (sunshine, h)		544	492	638	753	885	687	517	626
Total N	FP	91	96	75	103	83	101	87	73
	BF	51	66	39	51	44	51	59	50
	%R	40	31	37	52	39	51	28	23
Total P	FP	64	50	55	54	62	57	68	58
	BF	39	50	55	53	65	56	67	57
	%R	25	0	0	1	−2	1	1	1
Total K	FP	49	30	59	43	72	32	63	42
	BF	31	30	59	44	69	32	66	42
	%R	17	0	0	−1	3	0	−3	0

management system, rather than the biofertilizer per se, as it did not explicitly account for any differences in chemical fertilizer applied together with the biofertilizer.

Model 1:

$$\text{Yield} \sim \text{TRT} + \text{ENV} + \text{TRT} \times \text{ENV} + \text{ENV(FARM)}$$

Where

TRT	Two levels (biofertilizer practice and conventional practice)
ENV	Eight levels (season × locality)
ENV (FARM)	Farm nested in environment

We subsequently repeated the statistical analysis but included total N fertilizer application as a covariate in order to account for differences in farmer practices (model 2). Interactions between N fertilizer application and the other categorical factors were also considered; however, because TRT (i.e., ± biofertilizer) was not independent from TN (farmers were required to reduce TN if biofertilizer was applied), we did not include TRT×TN interaction in the model. We also tested the likely possibility of a nonlinear response to N fertilizer application rate by initially including a TN×TN term. Nonsignificant terms were sequentially dropped from the model in a backward-fitting procedure. The resulting best-fit model allowed for an estimation of the effect of the biofertilizer, regardless of chemical fertilizer, by controlling for the yield response to N fertilizer.

Model 2:

$$\text{Yield} \sim \text{TRT} + \text{ENV} + \text{TN} + \text{TRT} \times \text{ENV} + \text{ENV} \times \text{TN} + \text{TN} \times \text{TN} + \text{ENV(FARM)}$$

Where

TRT	Two levels (+biofertilizer, −biofertilizer)
ENV	Eight levels (season × locality)
TN	Total N fertilizer applied (covariate, kilograms per hectare)
ENV (FARM)	Farm nested in environment

To further investigate the performance of the biofertilizer management system over time, we conducted a regression stability analysis. Although this analysis is most commonly used to assess the yield stability of different genotypes through time under varying environmental conditions, it has also been applied to evaluate cropping systems and fertility management across environments (Grover et al. 2009). In regression stability analysis, an environment mean is calculated as the mean yield of all treatments being compared in a particular cropping season, and environments are then ranked by yield level to produce a quantitative gradient of environmental productivity irrespective of the cause of variability in yield (Hildebrand 1984). Individual treatments are subsequently regressed against the environment means, and the resulting linear fits are then compared amongst treatments. The assumption for stability analysis is that year-to-year variability in yield is due mainly to environmental variability; hence, change in yield over time should not differ amongst the treatments being compared. In our experiment, this assumption clearly does not hold, as optimization of the biofertilizer system (in terms of additional chemical fertilization) was deliberately allowed to evolve. Thus, the interpretation of our stability analysis precludes making any inferences about the *stability* of the biofertilizer system per se. Nevertheless, this type of analysis provides quick visualization in the change in performance of both biofertilizer and normal farmer practice over time with respect to each other.

Partial least squares regression was also applied to identify potential causes for variations in the efficacy of biofertilizer practice cf. conventional fertilizer practice. In particular, we wanted to determine how much variation in the performance of biofertilizer could be explained by the supplementary chemical fertilizer regime. Because all trials were conducted as split-plot experiments, a yield response ratio was calculated for each cropping season at each farm defined as the yield obtained from biofertilizer practice divided by the yield obtained from conventional practice.

General linear modeling and stability regressions were conducted using SPSS, IBM. Partial least squares regression was conducted using R (R development core team 2008) using the package 'mixOmics' (González et al. 2010).

3 Results and discussion

Microbial inoculants are being promoted for sustainable agriculture, but their uptake has been limited by unpredictable performance compared with chemical fertilizers under field conditions (Lucy et al. 2004). In this study, results from 20 different farms over four rice cropping seasons in the Mekong Delta highlight the challenges in using plant growth-promoting inoculants to consistently improve yields across multiple seasonal and spatial environments. However, the results also demonstrate that the likelihood of successful biofertilizer application can be increased if the seasonal crop nutrient requirements are understood and addressed.

3.1 Performance of the biofertilizer agronomy system

An important distinction to make when applying biofertilizers is the effect of the biofertilizer agronomy system (which may also include reductions in chemical fertilizers) versus the effect of the biofertilizer alone. We distinguished these effects by initially constructing a general linear model without covariates (model 1), which assessed the biofertilizer management system as a whole, and then repeating the analysis accounting for chemical fertilizers as covariates (model 2) in order to isolate biofertilizer effects.

The initial model showed significant ($P=0.001$) interactions between the growing environment (locality × season) and the fertilizer agronomy (Table 2, model 1). Although the mean yield at Cai Lay in the first season (late wet season, 2009) was significantly lower in biofertilizer management system plots than control plots (Table 3, Model 1), yields of biofertilizer-treated rice improved at both localities in the following three seasons. In these seasons, yields were equivalent to those of conventional practice, but with 23–52 % reductions of N fertilizer as shown in Table 1.

When the model was restructured with chemical fertilizers as covariates (Table 2, model 2), crop yield was significantly ($P=0.007$) affected by environment × N fertilization as well as environment × fertilizer agronomy interactions ($P<0.001$). Accounting for the environment × N fertilizer effect, the estimated yields of biofertilizer-treated rice were significantly greater than the yields of conventionally grown rice in three of the eight environments: Phung Hiep in the early wet season 2010 and both localities in the late wet season crop of 2010 (Table 3). The estimated average yield increases resulting from biofertilizer application in these environments was in the order of 17–22 %. This is similar to yield increases of 5–20 % reported in other extensive surveys involving different growth-promoting inoculants and crops such as the use of rhizobia in rice production in the Nile Delta (Yanni and Dazzo 2010), *Azospirillum* for wheat and maize production in Brazil (Hungria et al. 2010), and *Burkholderia* for rice production in Vietnam (Van et al. 2000) and India (Govindarajan et al. 2008). However, it must be noted that the biofertilizer per se had a negligible effect in the dry season crop (Table 3). This implies that reducing N fertilizer application in the dry season is a stronger driver of maintaining (or slightly improving) yields than the biofertilizer and vice versa for the late wet season.

To further examine the manner in which yields varied between the biofertilizer system and conventional practice, a stability analysis was conducted for each season. This showed that yield reductions under the biofertilizer system during the first cropping season (late wet season, 2009) were exacerbated when overall productivity was lower (Fig. 2, top left). The late wet season is the third cropping season when yields are much lower as a result of lowered fertility and pH in acid sulfate soils and lower light intensity. Nevertheless, the difference between the two systems could not be discerned in higher yielding plots in this season. The opposite (but not statistically significant) trend was observed for the dry season 2009–2010 in which the biofertilizer system generally performed better than the conventional counterpart under lower productivity conditions (Fig. 2, top right). No significant trend differences were apparent in the final two seasons, including the second lower yield season, showing the benefit of participatory experience.

The effects of environmental productivity on inoculant efficacy have been previously identified; however, some studies suggest that inoculant biofertilizers work best under higher fertility, while others indicate better performance under low productivity conditions. For example, Okon and Labanderagonzalez (1994) and Dobbelaere et al. (2001) found that beneficial effects of *Azospirillum* on crop yield were mainly observed under intermediate levels of fertilizer (N, P, and K) rather than maximum or minimum fertilization. Likewise, Diaz-Zorita and Fernandez-Canigia (2009) found that positive yield responses of wheat to liquid formulations of *Azospirillum* (at about 70 % of the sites) were mostly dependent on the site-specific attainable yield, with greater responses to inoculation generally observed in the absence of major crop growth limitations at sites of higher productivity. In contrast, a meta-analysis of 59 publications reporting the effects of *Azospirillum* inoculation on wheat showed that the

Table 2 General linear model statistical results for best-fit models assessing the effects of the biofertilizer management system (model 1, covariates excluded) and the biofertilizer itself (model 2, covariates and covariate interactions included)

Source	Model 1 (covariates excluded)		Model 2 (covariates included)	
	F	Sig.	*F*	Sig.
ENV × TRT	4.02	0.001	4.10	<0.001
TRT	Not significant		Not significant	
ENV	366.9	<0.001	7.90	<0.001
ENV (FARM)	6.70	<0.001	6.27	<0.001
TN	Not included		Not significant	
$(TN)^2$	Not included		Not significant	
ENV × TN	Not included		2.81	0.007

Table 3 Mean yield (tonnes per hectare) and confidence levels within environments estimated by two models assessing the effects of the biofertilizer management system (covariates excluded) and the biofertilizer itself (covariates included)

Environment	Fertilizer management	Model 1: without covariates		Model 2: with chemical N fertilizer level as covariate	
		Estimated yield (±95 % confidence level)	Yield increase/ decrease caused by biofertilizer system (%)	Estimated yield (±95 % confidence level)	Yield increase/decrease caused by biofertilizer (%)
Late wet season 2009: Cai Lay	Biofertilizer	*3.68±0.21*	*−11.3*	3.72±0.27	−8.4
	Farmer practice	*4.15±0.21*		4.06±0.63	
Late wet season 2009: Phung Hiep	Biofertilizer	4.05±0.23	−8.0	4.02±0.28	−13.2
	Farmer practice	4.40±0.23		4.63±1.34	
Dry season 2009–2010: Cai Lay	Biofertilizer	6.91±0.15	−0.6	6.71±0.75	−4.3
	Farmer practice	6.95±0.21		7.01±0.31	
Dry season 2009–2010: Phung Hiep	Biofertilizer	6.68±0.17	4.7	6.54±0.31	−3.4
	Farmer practice	6.38±0.22		6.77±0.75	
Early wet season 2010: Cai Lay	Biofertilizer	3.61±0.15	0.8	3.78±0.36	9.5
	Farmer practice	3.58±0.20		3.45±0.31	
Early wet season 2010: Phung Hiep	Biofertilizer	4.75±0.15	4.2	*4.98±0.57*	*22.1*
	Farmer practice	4.56±0.21		*4.08±1.21*	
Late wet season 2010: Cai Lay	Biofertilizer	4.60±0.18	6.7	*4.76±0.20*	*21.5*
	Farmer practice	4.31±0.16		*3.92±0.27*	
Late wet season 2010: Phung Hiep	Biofertilizer	3.87±0.17	5.4	*4.17±0.28*	*17.3*
	Farmer practice	3.67±0.15		*3.55±0.17*	

Italicized values indicate a significant difference between the two fertilizer management practices ($p<0.05$)

greatest increase yields over uninoculated controls occurred when additional N fertilizer was completely absent (Veresoglou and Menexes 2009). Shaharoona et al. (2008) also reported that the efficacy of *Pseudomonas* strains for improving growth and yield of wheat was highest with no additional NPK fertilizer, and the response declined with increasing rates of NPK. They suggested that under low fertilizer application, 1-aminocyclopropane-1-carboxylate-deaminase activity of certain plant growth-promoting rhizobacteria might inhibit the synthesis of stress (nutrient)-induced inhibitory levels of ethylene in the roots and lead to higher growth relative to control plants.

What is the cause of these discrepancies? In the case of the Mekong Delta, yields for the dry season crop are usually around 6 T ha^{-1} compared with 3–4 T ha^{-1} during the late wet season (Tan et al. 2004). Higher dry season crop yields are a consequence of renewed soil fertility after seasonal flooding combined with a greater number of sunlight hours (Tan et al. 2004). Plant nutrient availability and requirements of the dry season are therefore substantially different to both wet season crops. In the first trial season (a less productive late wet season crop), most farms had reduced chemical fertilizer rates, including lower P and K applications, in the biofertilizer plots. This resulted in significantly lower grain yields from plots receiving biofertilizer agronomy compared with those receiving full chemical fertilizer according to conventional farm practice. The stability regression for this first season indicated that the biggest difference existed on farms with lower general productivity, suggesting the need for a minimum level of fertility for the biofertilizer to contribute to plant growth. In the following seasons, when P and K fertilizer in biofertilizer plots was applied at similar rates to control plots, the relative yield of biofertilizer plots improved significantly. The linear regression model including N fertilizer as a covariate showed that the biofertilizer itself was responsible for yield increases of around 10–20 % when P and K were supplied in wet season crops, equivalent to N fertilizer reductions of 25 % or more without yield losses.

3.2 Importance of fertilizer applications and timing in the seasonal yield response

Because of the strong dependence of biofertilizer efficacy on seasonal productivity, we conducted partial least squares regression to determine how much variation in the yield response to biofertilizer could be explained by the supplementary chemical fertilizer regime. Chemical fertilizer management was least influential on biofertilizer efficacy in the dry season, explaining less than 30 % of the total variation. In this season, N application rate was the most important variable, with increasing application of N (relative to control plots) reducing the efficacy of the biofertilizer system, especially if more N was applied early in the season (Table 4). Although high rates of total N reduced yields under biofertilizer and

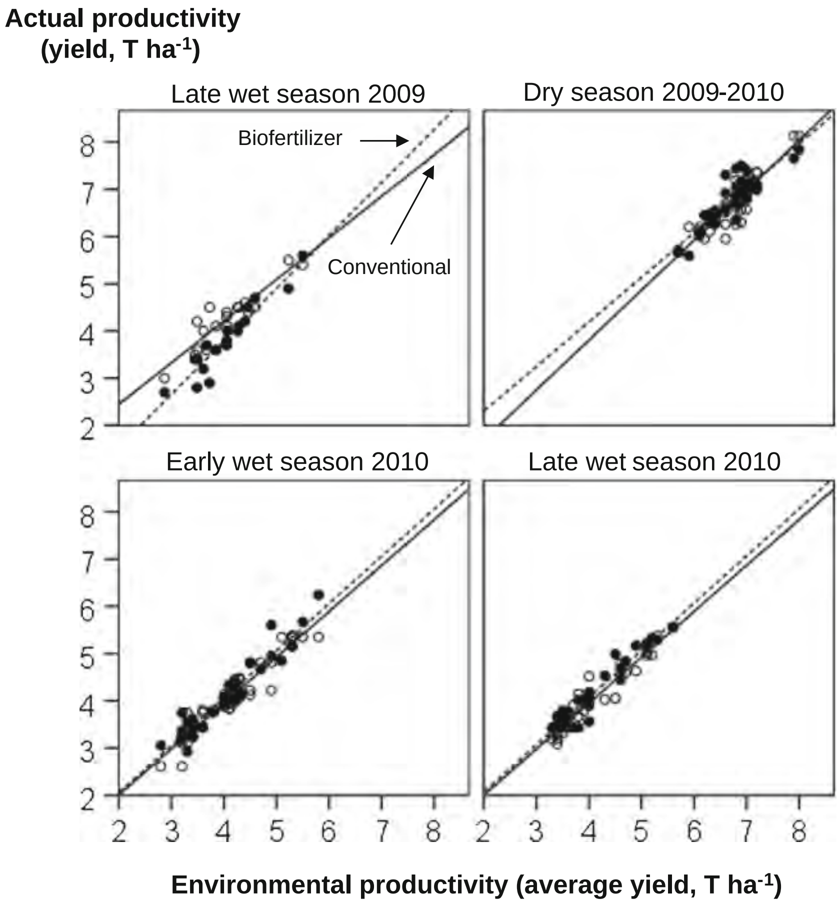

Fig. 2 Regression stability analysis for the biofertilizer system versus conventional practice

Table 4 Variable importance and loadings in the PLS regression, predicting biofertilizer yield response. Only variables with a variable importance in the projection >1 are shown. Positive loadings are related to increased biofertilizer yield relative to control yields; negative loadings are related to decreased biofertilizer yield relative to control yields

Cropping season	Predictor variable	Variable importance in the projection	Loadings		
			Component 1	Component 2	Component 3
Dry season (r^2=0.29; RMSE=0.065)	RTN	1.68	−0.53	0.11	0.21
	REN	1.43	−0.36	−0.48	−0.13
	ALN	1.33	0.39	−0.32	0.03
	ATK	1.33	−0.40	−0.27	0.07
	ATN	1.20	0.29	−0.43	−0.02
	AVGE	1.04	−0.16	0.07	−0.64
Early wet season (r^2=0.45; RMSE=0.088)	AEN	1.92	−0.64	−0.17	−0.08
	ALN	1.38	0.14	0.72	0.16
	ATN	1.22	−0.38	0.26	0.03
	AEP	1.13	−0.33	0.30	0.12
	REN	1.07	−0.31	−0.16	0.27
Late wet season (r^2=0.42; RMSE=0.092)	REP	1.46	0.47	0.03	0.19
	RLN	1.44	−0.28	−0.56	0.01
	RTP	1.29	0.39	−0.21	−0.10
	REN	1.29	0.40	−0.11	−0.24
	RTK	1.20	0.35	−0.24	0.12
	AEP	1.09	0.22	0.35	−0.43
	RLP	1.06	0.33	−0.06	0.20
	RTN	1.03	0.24	−0.32	0.30

Model fit parameters (r^2 and root mean square error (RMSE)) for each season are given in the first column

R/*A* relative/average, *E*/*L*/*T* early/late/total, *N*/*P*/*K* nitrogen/phosphorus/potassium farmer practice, the timing of application had a contrasting effect between the two systems. Yields under biofertilizer were negatively influenced to a greater extent by early N applications, whereas yield reductions under farmer practice were mainly a consequence of high rates of N fertilizer late in the season (Fig. 3, left column).

Supplementary fertilizer management had the greatest influence on the biofertilizer yield response in the early wet season, explaining up to 45 % of the variation. As with the dry season, high application rates of N early in the season reduced yields of biofertilizer-treated rice compared with farmer controls (Table 4, Fig. 3), but unlike the dry season, biofertilizer-treated rice yields were significantly improved by high application of N fertilizer late in the season (Fig. 3, center), leading to higher relative yields under these conditions.

In the late wet season, up to 42 % of the variation in yield response could be explained by the chemical fertilizer management, regardless of other site specific soil or environmental variables. In this season, the most important factors influencing the biofertilizer performance were early fertilizer and total P fertilizer and early N applications (Table 4). Higher application rates of P and early N were positively related to biofertilizer efficacy. In contrast to the other seasons, biofertilizer-treated rice (Fig. 3, bottom right) was also more responsive to total N application than farmer practice.

Together, our results support previous findings that BioGro biofertilizer is able to supplement or replace a certain proportion of chemical N fertilizer (Phan et al. 2009), but biofertilizer does not necessarily contribute to improve P nutrition (Cong et al. 2011). There is increasing evidence that N nutrition, not P nutrition, is more effectively enhanced by PGPRs under a wide range of conditions (de Freitas et al. 1997; Adesemoye et al. 2009; Adesemoye and Kloepper 2009). In addition to the amount and type of chemical fertilizer applied, our results also indicate that the timing of fertilizer applications can also have a strong effect on the yield of biofertilizer-treated rice. To our knowledge, the interaction of chemical fertilizer timing and biofertilizer efficacy has not yet been investigated or reported. Here, we found that although yields under both biofertilizer and farmer practice were negatively related to higher N fertilizer in the dry season, the timing of application had a contrasting effect between the two management systems (Fig. 3). Yields under biofertilizer were influenced more by early N applications, whereas yield reductions under farmer practice were mainly a consequence of high rates of N fertilizer late in the season. We hypothesize that the high soil fertility in the dry season immediately following flooding favors strong seedling establishment and vigor, thus providing a suitable environment for microbial inoculant survival and root colonization without additional chemical fertilizer. Indeed, increased early chemical N fertilizer appears to inhibit plant growth

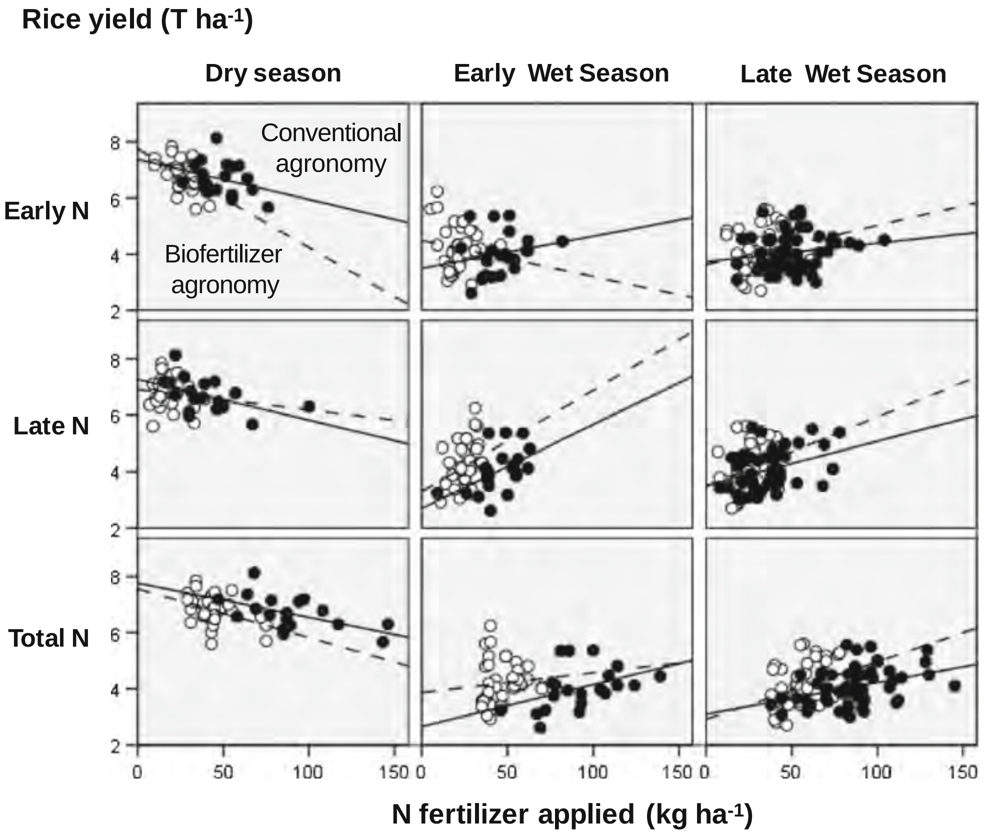

Fig. 3 Rice yield response (tonnes per hectare) to N fertilizer timing and total N under biofertilizer (*open circles*, *dashed line*) or conventional agronomy (*closed circles*, *solid line*) in different seasons. Linear regressions have been fit to aid visualization, but note that such trends should be interpreted only within the ranges of N application to which they are actually applied. Regression fit parameters (r^2, p value) are available in Supplementary file 3

promotion. In contrast, for the late wet season crop, biofertilizer performance was greatest when early N and P applications were supplied at high rates and late N applications were restricted. Because soil nutrients in the second wet season are relatively depleted at sowing, it appears that a greater fertilizer requirement is necessary for early plant growth and microbial establishment.

3.3 Benefits and limitations of using a farmer participatory approach

The benefits of conducting farmer participatory trials go beyond addressing the original experimental research aims by harnessing farmer observations that might otherwise be neglected by traditional research methods. Firstly, by involving farmers directly in the research program, farmers take ownership of technology, optimizing to their own conditions and preferences. On a local level, this involvement encourages fast technology uptake by improving confidence and also provides a greater knowledge base for regional transfer of skills. Secondly, farmers provide critical feedback to researchers about applying the new technology in terms of on-the-ground practicalities that may have been overlooked in laboratory-scale settings, being more aware of risks to their profitability. Thirdly, farmers are more likely to identify additional subtle effects of the technology that could be missed by researchers by virtue of (usually) years of experience in crop agronomy at the site level. For example, farmers involved in this research project reported a reduced need for pesticides in biofertilizer-treated rice because of perceived greater resistance to attack. Such anecdotal feedback was borne out in the economic data collected, showing reduced pesticide applications in biofertilizer-treated rice (data not shown). Farmers also reported better visual grain quality, but this could not be validated at the time by analytical testing and thus cannot be scientifically supported/rejected at this stage.

Nevertheless, there are some limitations to the farmer participatory approach that should be acknowledged. In our research, reluctance amongst farmers to maintain multiple treatment plots together with prohibitory research costs meant that full chemical fertilizer response curves could not be ascertained for individual farms. Consequently, N fertilizer response could only be described by linear regressions despite the well-known fact that yield curves are usually better described by quadratic or modified exponential functions. Furthermore, farm-specific interactions between chemical fertilizer and biofertilizer could not be modeled (as outlined in the Methodology section) even though such interactions are known to exist (Phan et al. 2009). Finally, it is almost certain that seasonal variations in soil properties would have affected the yield response, but such in-depth soil monitoring could not be carried out because of the high costs associated with sampling and analysis over such a large scale. Together, these drawbacks result in a decreased resolution of the site-specific interactions between chemical and biological fertilizers that regulate crop yields and economic returns. In the future, where the resources are available, a more complete factorial design at a reduced number of sites together with comprehensive monitoring of soil properties would help overcome these limitations.

4 Conclusions

There is a large knowledge gap on why inoculant biofertilizers may or may not work under field conditions spanning multiple spatial and seasonal scales. This study has shown that:

- The efficacy of a commercial biofertilizer is strongly dependent on seasonal and site-specific environmental conditions.
- Up to 45 % of the variation in the biofertilizer effect could be ascribed to differences in the timing and magnitude of chemical fertilizers applied simultaneously to the growing crop. Such variation can therefore be managed in order to minimize farmers' risk in adopting the technology. Importantly, the biofertilizer could replace between 23 and 52 % of N fertilizer without loss of yield but did not appear to be able to replace P or K fertilizer.
- A farmer participatory approach to the application of biofertilizers enabled rapid optimization under field conditions, which in turn increased farmer confidence and the reproducibility of agronomic benefits.

Such information will accelerate the practical adoption of biofertilizers into cropping practices by addressing current knowledge gaps that exist between laboratory and field scales. The outcome will be a more sustainable rice production system through a reduced reliance on high inputs of chemical fertilizers.

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