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Title: Evaluation of rice-legume-rice cropping system on grain yield, nutrient uptake, nitrogen fixation, and chemical, physical and biological properties of soil.

Article Type: Original Paper

Keywords: Azospirillum ☐ Compost ☐ DGGE ☐ Fungal/bacterial biomass-C ratio ☐ N balance ☐ P balance ☐ Phosphate solubilizing bacteria ☐ Rhizobium ☐ Zn balance

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Abstract: To achieve higher yields and better soil quality under rice-legume-rice (RLR) rotation in rainfed production system, we formulated integrated nutrient management (INM) comprised of Azospirillum (Azo), Rhizobium (Rh), phosphate solubilizing bacteria (PSB) with phosphate rock (PR), compost and muriate of potash (MOP). Performance of bacterial bioinoculants was evaluated by determining grain yield, nitrogenase activity, uptake and balance of N, P and Zn, changes in water-stability and distribution of soil aggregates, soil organic C and pH, fungal/bacterial biomass C ratio, casting activities of earthworms and bacterial community composition using denaturing gradient gel electrophoresis (DGGE) fingerprinting. The performance comparison was made against the prevailing farmers' nutrient management practices [N:P2O5:K2O @ 40:20:20 kg ha-1 for rice and 20:30:20 kg ha-1 for legume as urea:single-superphosphate:MOP (Urea:SSP:MOP)]. Cumulative grain yields of crops increased by 7-16% per RLR rotation and removal of N and P by six crops of 2 years rotation increased significantly ($P<0.05$) in bacterial bioinoculants based INM

plots over that in compost alone or Urea:SSP:MOP plots. Apparent loss of soil total N and P at 0-15 cm soil depth was minimum and apparent N gain at 15-30 cm depth was maximum in Azo/Rh plus PSB dual INM plots. Zinc uptake by rice crop and diethylenetriaminepentaacetate extractable Zn content in soil increased significantly ($P<0.05$) in bacterial bioinoculants based INM plots compared to other nutrient management plots. Total organic C content in soil declined at 0-15 cm depth and increased at 15-30 cm depth in all nutrient management plots after 2 years crop cycles; however, bacterial bioinoculants based INM plots showed minimum loss and maximum gain of total organic C content in the corresponding soil depths. Water stable aggregation and distribution of soil aggregates in 2000-250 □ m and 250-53 □ m classes increased significantly ($P<0.05$) in bacterial bioinoculants based INM plots compared to other nutrient management plots. Fungal/bacterial biomass-C ratio seems to be more reliable indicator of C and N dynamics in acidic soils than total microbial biomass-C. Compost alone or Azo/Rh plus PSB dual INM plots showed significant ($P<0.05$) higher numbers of earthworms' casts compared to Urea:SSP:MOP alone and bacterial bioinoculants with urea or SSP applied plots. Hierarchical cluster analysis based on similarity matrix of DGGE profiles revealed changes in bacterial community compositions in soils due to differences in nutrient managements, and these changes were seen to occur according to the states of C and N dynamics in acidic soil under RLR rotation.

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Author's response to reviewers' comments_R2

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In the following pages, the Editor's comments are in italics, followed by details of changes/modifications or responses from the authors (in plain type). Please note that page and line numbers refer to the revised version.

1. Editor comments

I have read your revised manuscript titled "Evaluation of bacterial bioinoculants for use as components of integrated nutrient management in sustaining rainfed rice-legume-rice cropping system" and it needs a further revision according to the comments:

We thank the editor-in-chief for these encouraging comments. We took actions against all suggested comments as follows:

Please modify the title as "Evaluation of.....croppimg system on grain yoeld, nutrient uptake, nitrogen fixation, and chemical, physical and biological proprties of soil".
p. 1, Line 7-9: now stated that
"**Evaluation of rice-legume-rice cropping system on grain yield, nutrient uptake, nitrogen fixation, and chemical, physical and biological properties of soil."**

Line 102-Please write "Yadvinder-Singh et al 2004;"
p. 4, Line 102: now stated that
"….; Yadvinder-Singh et al. 2004; Reddy and Raju 2006; Pampolino et al. 2007)."

Lines 105-106-Please write "being a N rich grain".
p. 4, Line 105-106: now stated that
"Besides being a N rich grain,...."

Line 295-please write "of excess NaOH and ammonia".
p. 11, Line 295: now stated that
"distillation in presence of excess NaOH and ammonia……."

Line 296-297-Please write "boric acid; these steps were done by using Kjel.....India. Then residual boric acid was titled with standard...HClO4, 3:1, as described".
p. 12, Line 296-298: now stated that
"…boric acid; these steps were done by using Kjel Plus, Pelican Equipments, India. Then residual boric acid was titrated with standard 0.01 N H_2SO_4 and total P by digestion in HNO_3:$HClO_4$, 3:1, as described (Olsen and Sommers 1982)."

Line 300-Please write "(DTPA) as described by".
p. 12, Line 300: now stated that
"…by using DTPA as described by Liang and Karamanos (1993)……."

Lines 311-312-Please write "Bacterial and fungal counts of soil were".
p. 12, Line 312: now stated that
"Bacterial and fungal counts of soil were determined …."

Line 348-Please write "were maintained as described by".
p. 14, Line 348: now stated that
"….reaction conditions were maintained as described by Muyzer et al. (1993)."

Line 395-Please write "low organic C status".
p. 16, Line 395: now stated that
"….low organic C status (<8.0 g kg^{-1} soil)….."

Line 419-Please write "that of other treatment".
p. 16, Line 419: now stated that
"….that of other treatments."

Line 508- The citation is "Kucey et al".
p. 20, Line 508: now stated that
"….mediated mechanisms (Kucey et al. 1989)…"

Lines 520-521-Please write "Sali rice 2002 are presented".
p. 21, Line 520-521: now stated that
"….*Sali* rice 2002 are presented in the Table 4…"

Line 549-Please write "1986). Nakling et al".
p. 22, Line 549: now stated that
"….(Dalal and Mayer 1986). Earlier, Naklang et al. (1999) observed ……"

Lines 554-563-Please write "and mass of 2000-250 um and 250-53 um soil aggregates at 0-15 cm.....(Table 5. Mass of soil in aggregates class <53 um increased significantly (P<0.005) in control.....and compost plots compared to that in bacterial bioinoculants based INM plots. The formation and stabilization.....deeper soil depths. These macro- and micro-aggregates contain higher".
p. 22, Line 553-564: now stated that
"Bacterial bioinoculants based INM plots showed significant (P<0.05) higher water-stable aggregation and mass of 2000-250 μm and 250-53 μm soil aggregates at 0-15 cm soil depth compared to that in control, Urea:SSP:MOP and compost alone plots after harvest of six crops in RLR rotation (Table 5). Mass of soil in aggregates class <53 μm increased significantly (P<0.05) in control, Urea:SSP:MOP and compost plots compared to that in bacterial bioinoculants based INM plots. The formation and stabilization of macro-aggregates (250-2000 μm) and micro-aggregates (53-250 μm) in these plots perhaps physically protected higher amount of particulate organic matter and hence, less chance of depletion of labile organic C from the surface layer (0-15 cm depth) to deeper soil depths. These macro- and micro-aggregates contain higher amounts of particulate and light fraction organic matters and that support higher rate of C and N mineralization in soils (Manna et al. 2005; Yan et al. 2007)."

Lines 606-613.Please summarise and be simple and clear. I do not understand the meaning of sentences at lines 610-613.

p. 24, Line 605-609: now stated that
"Overall, results of FBC/BBC ratios suggested that incorporation of legume and rice crop residues into soils along with external application of compost and bacterial bioinoculants under rice based rotation were useful in maintaining balance between C and N dynamics in soils. A balance between C and N dynamics in soils ensures more labile pools of soil organic C, and hence better mineralization processes of nutrients in soils."

Lines 614-615-Please write "earthworms' castings approximately doubled (significant at P".
p. 24, Line 610-611: now stated that
"……earthworms' castings approximately doubled (significant at $P<0.05$) in Azo/Rh plus PSB dual INM….."

Line 625-Please write "or SSP reduced casting".
p. 25, Line 620-621: now stated that
"……addition of either urea or SSP reduced casting activities of earthworms in Azo/Rh alone INM, ..……"

Lines 647-655. All this part is confusing. I do not understand the meaning of the sentence at lines 647-649. I suggest deleting the sentence at lines 653-655 and rewrite in a simple, clear and short way the rest of the text.
p. 25-26, Line 643-650: now stated that
"Interestingly, C and N dynamics in soils between Azo/Rh plus PSB dual INM and Azo/Rh alone INM plots were comparable, and soils of these two plots also harboured highly similar bacterial communities. Like-wise, C and N dynamics, bacterial community compositions in soils between Urea:SSP:MOP and urea added PSB alone INM plots were comparable. These findings implied that nutrient inputs such as legume and rice crops residues, bacterial bioinoculants, inorganic fertilizers added in different combinations to nutrient management plots modified community composition of soil bacteria through their direct influence on C and N dynamics."

Is the reference at line 788 cited in the text? Yes, it is cited in the text as follows
p. 17, Line 422:
"….observation by earlier workers (Roper and Ladha 1995), we also found that…"

In the 4 figure legends you have to include the meaning of acronyms. I suggest writing "Azo is...; Rh is ...etc".
p. 33-34, Line 837-863: now stated that

"Fig. 1 Harvest index of rice crops influenced by different nutrient management treatments in rice-legume-rice rotation. Values that differ significantly (one-way ANOVA, $P<0.05$) within each cluster of dendrograms are followed by different letters. Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Fig. 2 Nitrogenase activity in roots of *Sali* rice (A), pea (B) and *Ahu* rice (C) influenced by different nutrient management treatments under rice-legume-rice rotation. Nitrogenase activity in roots of *Sali* rice and French bean of 1st year crop cycle were not determined.

Each value on the line graph represents mean nitrogenase activity in roots of 12 plants from four replicated plots. Values that differ significantly (one-way ANOVA, $P<0.01$) on each line graph are followed by different letters. Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Fig. 3 Microbial biomass C (MBC), bacterial biomass C (BBC) and fungal biomass C (FBC) influenced by different nutrient management treatments determined after harvest of six crops in rice-legume-rice rotation. Values that differ significantly (one-way ANOVA, $P<0.05$) within each parameter are followed by different letters. Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Fig. 4 Denaturing gradient gel electrophoresis (DGGE) profiles of 16S rRNA gene fragments obtained by PCR amplification using bacterial primer sets (Muyzer et al. 1993) in soils of different nutrient management treatments. (A) an image of ethidium bromide stained DGGE gel and (B) hierarchical cluster plot based on similarity matrix of DGGE profiles. Joints of the branches of the dendrogram indicate the percentage similarity based on unweighted pair group method with arithmetic means (UPGMA). Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash."

Table 1 Please write "Crop cycle and year"
Now stated that
"**Table 1** Crop cycle and year, fertilizer application rate and form applied to nine crops in rice-legume-rice rotation during 2001-2004"

Add at each of the 5 tables as a footnote: "SSP is....; MOP is...; PR is...etc".
Table 1 foot-note included the following:
"PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash."
The following sentence was included in foot-note of each of the Tables from 2 to 5.
"Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash."

p. 27, Lines 672-674:
Now acknowledgement section stated as:

"Acknowledgements We thank the Department of Biotechnology, Ministry of Science and Technology, Government of India for financial support to carry out this research. We also thank Paolo Nannipieri and Kazuyuki Inubushi for critical review of the manuscript."

ENDS

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Abstract To achieve higher yields and better soil quality under rice-legume-rice (RLR) rotation in rainfed production system, we formulated integrated nutrient management (INM) comprised of *Azospirillum* (Azo), *Rhizobium* (Rh), phosphate solubilizing bacteria (PSB) with phosphate rock (PR), compost and muriate of potash (MOP). Performance of bacterial bioinoculants was evaluated by determining grain yield, nitrogenase activity, uptake and balance of N, P and Zn, changes in water-stability and distribution of soil aggregates, soil organic C and pH, fungal/bacterial biomass C ratio, casting activities of earthworms and bacterial community composition using denaturing gradient gel electrophoresis (DGGE) fingerprinting. The performance comparison was made against the prevailing farmers' nutrient management practices [$N:P_2O_5:K_2O$ @ 40:20:20 kg ha^{-1} for rice and 20:30:20 kg ha^{-1} for legume as urea:single-superphosphate:MOP (Urea:SSP:MOP)]. Cumulative grain yields of crops increased by 7-16% per RLR rotation and removal of N and P by six crops of 2 years rotation increased significantly ($P<0.05$) in bacterial bioinoculants based INM plots over that in compost alone or Urea:SSP:MOP plots. Apparent loss of soil total N and P at 0-15 cm soil depth was minimum and apparent N gain at 15-30 cm depth was maximum in Azo/Rh plus PSB dual INM plots. Zinc uptake by rice crop and diethylenetriaminepentaacetate extractable Zn content in soil increased significantly ($P<0.05$) in bacterial bioinoculants based INM plots compared to other nutrient management plots. Total organic C content in soil declined at 0-15 cm depth and increased at 15-30 cm depth in all nutrient management plots after 2 years crop cycles; however, bacterial bioinoculants based INM plots showed minimum loss and maximum gain of total organic C content in the corresponding soil depths. Water stable aggregation and distribution of soil aggregates in 2000-250 μm and 250-53 μm classes increased significantly ($P<0.05$) in bacterial bioinoculants based INM plots compared to other nutrient management plots. Fungal/bacterial biomass-C ratio seems to be more reliable indicator of C and N dynamics in acidic soils than total microbial biomass-C. Compost alone

or Azo/Rh plus PSB dual INM plots showed significant (P<0.05) higher numbers of earthworms' casts compared to Urea:SSP:MOP alone and bacterial bioinoculants with urea or SSP applied plots. Hierarchical cluster analysis based on similarity matrix of DGGE profiles revealed changes in bacterial community compositions in soils due to differences in nutrient managements, and these changes were seen to occur according to the states of C and N dynamics in acidic soil under RLR rotation.

Keywords *Azospirillum* · Compost · DGGE · Fungal/bacterial biomass-C ratio · N balance · P balance · Phosphate-solubilizing bacteria · *Rhizobium* · Zn balance

Introduction

To increase crop productivity under rainfed rice cropping systems in sustainable manner, efficient nutrient management approach needs to be developed keeping in view the factors of low productivity inherent in the systems. In northeastern alluvial plains of India, factors of low productivity of rice are (a) the nutrient content in soil and their use efficiency (NUE) is low, for example highly weathered light texture alluvium soils of Brahmaputra basin are prone to intense leaching losses of applied nitrogenous fertilizers coupled with high (> 81%) fixation rate of applied phosphatic fertilizers due to high activities of Fe and Al oxides, and Zn deficiency; (b) lack of site-specific efficient nutrient management approach; (c) low soil organic carbon (SOC) content (<8.0 g kg^{-1}); (d) uneven distribution of rainfall throughout crop growing periods; (e) no or little inorganic fertilizers ($N:P_2O_5:K_2O$) use @ 13 kg ha^{-1} in the northeastern region of India and (f) poor economic condition of farmers (Tewari et al.

1969; Talukdar and Chakravarty 1988; Khan et al. 2004; Talukdar et al. 2004). Under such conditions, increasing cropping intensity from double to triple in a year, without affecting soil quality is a major challenging task. This demands that several different aspects including cultivation of right crop in rotation with rice, recycling of crop residues and efficient nutrient management approach inclusive of different sources of nutrients are addressed through systematic research. Combined application of inorganic fertilizers with blue-green algae or green manure or farmyard manure with or without crop residue incorporation is known to improve NUE and higher yields in rice-based cropping systems (Regmi et al. 2002; Yadvinder-Singh et al. 2004; Reddy and Raju 2006; Pampolino et al. 2007).

Inclusion of legume crop in rotation is an important aspect of N and C management in fragile soils (Ladha and Reddy 2003) and also an opportunity to meet the perpetuated deficit in per capita availability of pulses in India (Prasad and Nagarajan 2004). Besides being a N rich grain, legume crop can also serve the role of green manure in the triple cropped rice systems by contributing N and biomass to the soil (George et al. 1994; Dobermann and White 1999; Yadav 2003). Application of inorganic N fertilizer at higher rate to boost crop productivity in acidic soils under rainfed rice systems is not a profitable N management approach due to very low N use efficiency. A recent study has indicated that use of inorganic N fertilizer at rate exceeding grain N removal caused a net decline in soil C despite increasingly massive residue C incorporation (Khan et al. 2007). Therefore, we presumed that any N management strategy that involves higher rate of inorganic N fertilizer application together with crop residue incorporation into soil to boost high yields from rainfed rice systems would be a suicidal approach. In this context, concept of integrated nutrient management (INM) might be fruitful in increasing grain yields in triple cropped rice-legume-rice (RLR) rotation and also in sustaining soil productivity and overall environmental quality (DeDutta 1989; Yadav 2003; Pampolino et al. 2007). Use of bacterial bioinoculants such as

azospirilla or rhizobia with phosphate solubilizing bacteria and phosphate rock (a slow release mineral P source) with compost and crop residue incorporation might increase NUE of major limiting nutrients N, P and Zn including better management of soil C under RLR rotation in acidic soils (Ladha and Reddy 2003; Somado et al. 2003; Choudhury and Kennedy 2004). Many previous studies confirmed the benefits of single or dual inoculation of phosphate solubilizing bacteria with azospirilla or rhizobia to cereals and legumes (Jeyabal and Kuppuswamy 2001; Johri et al. 2003; Somado et al. 2003; Choudhury and Kennedy 2004; Lucy et al. 2004; Reddy and Raju 2006). However, data on performance (in terms of grain yield, nutrient balance and soil quality) of single or dual inoculation of these beneficial microorganisms as components of INM in acidic rice soils under RLR rotation and residue incorporation over several seasons are limited.

The objective of this study was to determine performance of bacterial bioinoculants (*Azosprillum*, *Rhizobium* and phosphate solubilizing bacteria) based INM treatments against the existing farmers' nutrient management practices ($N:P_2O_5:K_2O$ @ 40:20:20 kg ha^{-1} for rice and 20:30:20 kg ha^{-1} for legume) for RLR rotation in acidic alluvial soils of northeastern plains of India in order to achieve higher productivity and soil sustainability. The performance comparison was done in terms of grain yields, uptake and balance of N, P and Zn and changes in organic C, aggregation, bacterial and fungal biomass C, casting activities of earthworms in soil. We also assessed the impacts of continuous application of bacterial bioinoculants based INM on composition of bacterial communities of soils under different nutrient managements in RLR rotation.

Materials and methods

Experimental location and climate

A field experiment was set-up at the experimental farm of Assam Agricultural University ($24^0 46^/$ N, $94^0 13^/$ E and 87 m above mean sea level) located in Assam, India. The field was not cultivated in the last 5 years prior to this experiment. Climate of the region is typic sub-tropical humid and receives mean annual rainfall 1931 mm and average rainy days 157 per annum. Total bright sunshine hour (BSSH) is 2129 hours against maximum possible BSSH of 4432 hours per year. Mean relative humidity is 79%. During experimental years 2001–2004, the mean maximum and minimum temperatures recorded during *Sali* rice (*Kharif*, August-November) seasons were 30.6 and 21.7 ^{0}C, legume (*Rabi*, December-March) seasons were 25.0 and 11.0 ^{0}C and *Ahu* rice (*Summer*, April-July) were 30.9 and 21.6 ^{0}C, respectively. The length of crop growing period (LGP) is >210 days in a year in this agro-ecological zone.

Plot layout, soil characteristics, crops and treatments

The Experimental field was divided into six blocks. Each block represented one nutrient treatment (see below for treatments detail) and within each block, four plots were the replicates each with an area of 4 x 5 m^2. Each block was laterally isolated by polythene sheets embedded into the soil to a depth 30 cm. The experiment was arranged as completely randomized block design. An uniformity trial of soil fertility on the experimental field was carried out before the start of the RLR rotation crops by growing high yielding *Ahu* rice (*Summer* rice) variety 'Luit' in close spacing (10 cm x 10 cm, between rows x plants).

The initial soil characteristics of the experimental field were determined after completion of the uniformity trial. The sandy loam inceptisol (Oxyaquic Dystrocrept) had the following properties: sand 55%, silt 30%, clay 15%, bulk density 1.36 Mg m^{-3}, pH (1:2,

soil:water) 4.80, total organic C 8.8 g kg^{-1} soil, total N 1.07 g kg^{-1} soil, total P 210 mg kg^{-1} soil, diethylenetriaminepentaacetate (DTPA) extractable Zn 0.62 mg kg^{-1} soil, cation exchange capacity 3.17 cmol kg^{-1} soil, base saturation 65.5% and water holding capacity 372 g kg^{-1} soil.

Nine crops in 3 years crop cycles were successfully harvested. The year-wise crop calendar is presented in Table 1. For both *Sali* and *Ahu* rice, three rice seedlings together (25 days old) were transplanted in the puddled plots at spacing 30 cm x 10 cm (between rows x between plants). French bean (*Phaseolus vulgaris* L.), the grain legume of the 1st year crop cycle, was sown at spacing 35 cm x 15 cm (between rows x between plants) after harvest of the first *Sali* rice crop of the experiment following land preparation. In 2nd and 3rd year crop cycle, pea (*Pisum sativum* L.) was grown as relay crop with *Sali* rice (Palaniappan 1985). Twenty-five days before harvest of *Sali* rice, pea seeds were sown in the inter-row spaces of *Sali* rice at spacing 30 cm x 10 cm (between rows x between plants).

Bioinoculants used were: *Azospirillum amazonense* A10 (MTCC 4716), *Rhizobium phaseoli* (FB-9-2) for French bean or *Rhizobium leguminosarum* $AAURh_1$ for pea and *Bacillus megaterium* P5 (MTCC 4714) as phosphate solubilizing bacteria and hereafter referred to as Azo, Rh and PSB, respectively (Thakuria et al. 2004). Compost was prepared from farm waste (N–16.7 g kg^{-1}, P–2.6 g kg^{-1} and K–8.8 g kg^{-1}). The six different nutrient management treatments were: 1. Control (no addition of compost, inorganic fertilizers and bioinoculants), 2. N:P_2O_5:K_2O applied as urea:single super-phosphate:muriate of potash, hereafter referred to as Urea:SSP:MOP, 3. Compost alone, 4. Compost + Azo (for rice) or Rh (for french bean/pea) + PSB + phosphate rock (PR) + MOP (hereafter referred to as Azo/Rh plus PSB dual INM), 5. Compost + Azo/Rh + SSP + MOP (hereafter referred to as Azo/Rh alone INM) and 6. Compost + urea + PSB + PR + MOP (hereafter referred to as PSB alone INM). The half amounts of the recommended quantity of urea (inorganic N) and the whole

amounts of the recommended quantities of SSP (inorganic P) and MOP (inorganic K) were applied in the puddled plots as basal application one day before transplanting of rice seedlings and the remaining half quantity of urea was top dressed on standing rice crop after 30 days of transplantation (DAT). Urea, SSP and MOP were applied as basal dose to the legume crops in rows 2 days before seeding. Compost was applied to respective treatment plots 10 days before transplanting in case of rice or at the time of seeding in case of pea. Crop-wise fertilizer dose applied to nine crops during 3 years crop cycles are presented in Table 1.

Dry compost (particle size <1 mm) in 500 g packets were double sterilized (at 121 ^{0}C under 0.11 MPa for 15 min twice at 36 h interval) to ensure complete sterilization and used as carrier material for bioinoculants Azo, Rh and PSB. Known quantity of broth culture of each bioinoculant was mixed separately with sterilized compost as described by Thakuria et al. 2004. The number of cells of Azo, PSB and Rh were 3.5 x 10^{9}, 3.3 x 10^{8} and 2.9 x 10^{9} *cfu* g^{-1} compost, respectively. The compost-based Azo, Rh and PSB bioinoculants were applied (@ 4 kg ha^{-1}) to rice seedlings by root-dip technique. The required quantity of bioinoculants was made into slurry and the rice seedlings of respective treatments were dipped for 3 h prior to transplanting. By this technique the bioinoculants were adhered to the seedling roots. After root-dip treatment of rice seedlings, the average population of Azo and PSB determined on inoculated rice seedling roots were 8.3 x 10^{7} *cfu* on rojo congo agar (Cáceres 1982) and 7.8 x 10^{6} on Pikovskaya's agar (Sundara Rao and Sinha 1963), respectively. Pea seeds were coated with Rh and average *cfu* per coated seed determined on yeast extract mannitol agar (Subba Rao 1999) was 6.9 x 10^{6}. French bean seeds were coated with *Rhizobium phaseoli* (FB-9-2) and *cfu* on seeds were not quantified.

Crop harvesting and residue recycling

Both *Sali* and *Ahu* rice were harvested at physiological maturity stage. Pods of french bean and pea crops were picked up thrice in sequence. French bean pods were harvested as green vegetable, whereas the pea was harvested as mature pods. *Ahu* rice straw and legume stover were harvested just at level to the soil surface. The fresh rice straw and legume stover from each plot were weighed and a uniform sample of 2 kg (rice) and 1 kg (legume) withdrawn, oven-dried at 65 ^{0}C to constant weight and weighed. Oven-dry weight of the sample was used to convert the fresh straw and stover weight on oven-dry basis. The remaining portion of the straw and stover was again immediately incorporated into the soil of respective plots. In case of *Sali* rice of 2nd and 3rd year rotations, panicles were harvested for grain yield and the straw yield was estimated by cutting only 10 hills at ground level in uniform pattern from each plot.

Nutrient balance in soil after harvest of six crops (2 years rotation)

The depth-wise (at 0-15 cm and 15-30 cm) nutrient balance sheets in soil were calculated at the end of second year rotation. Straw and stover of rice and legume crops were incorporated into soil and hence, nutrient removed by six crops referred to the grain nutrient uptake by six crops plus nutrient uptake by straw of the *Ahu* rice 2003 i.e. the sixth crop. In this study, the possibilities of inputs error through rice seedlings, legume seeds and also from rainfall to nutrient treatment plots were negligible for nutrient balance calculation, because each treatment plot received equal numbers of rice seedlings (same age) and legume seeds as planting materials and also equal amount of rainfall. During dry spell in each *Ahu* rice season, one life saving irrigation was given in equal volume from the same irrigation source to all treatment plots and it was assumed that any nutrient added to the plots through irrigation water was in equal amount and did not affect nutrient balance results. There was no flood on the plots during the *Sali* (monsoon) seasons of experimentation period and thus assumed to

have no nutrient loss by run off from the plots. The nutrient balance under these sets of experimental conditions indicated apparent loss or gain of N and P balances to make relative comparison among different nutrient management treatments. Depletion of DTPA extractable Zn was determined after completion of 1st year crop cycle. Apparent N and P balances were estimated using the method described by Regmi et al. (2002). We didn't consider N and P inputs through irrigation and rain waters in the balance estimation.

N or P balance = ∑ (N or P from compost & inorganic fertilizers) – plant N or P (uptake in grain & straw or stover)

Determination of nitrogenase activity by acetylene reduction assay

Closed acetylene reduction assay (ARA) can accurately indicate relative differences in nitrogenase activity in legume root nodules, though total nitrogenase activity measurement is not possible (Vessey 1994). ARA was determined in pea roots collected when maximum nodules were observed, and in rice roots collected at maximum tillering stage. Pea plants were uprooted and excess adhered soils were removed carefully. Entire roots with intact nodules of each plant were put in a glass bottle (volume 630 ml) and mouth was made airtight with rubber septum and 10% of the bottle's air space replaced by acetylene gas (C_2H_2, >99.99% purity) and incubated at room temperature for 1 h. For determination of nitrogenase activity in rice roots, the entire roots of a rice hill was uprooted and separated from the above ground plant parts. The entire root was rinsed with standing water on same spot in the field to remove excess adhered mud and immediately placed in a glass bottle (volume 630 ml) and mouth was made airtight with rubber septum. An air volume of 10% of the bottle's air space was replaced by injecting acetylene gas (C_2H_2, >99.99% purity). Bottles were incubated at room temperature for 16 h at dark (Barraquio et al. 1986). Ethylene production was measured on a gas chromatogram (GC Top series 8000, CE instruments, Italy) by standard procedure and

nitrogenase activity expressed in μmole of C_2H_4 h^{-1} 100 cc^{-1} root volume (for rice) and μmole of C_2H_4 h^{-1} $plant^{-1}$ (for pea) (Thakuria et al. 2004).

Soil and plant sampling and analyses

Soil samples (moisture content at field capacity) were collected randomly from 10 spots within each treatment plot up to 0-15 and 15-30 cm soil depth using a 5 cm diameter soil core at the end of both 1 and 2 year crop cycles (*Ahu* rice harvest). Each soil sample of 0-15 cm depth was divided into three sub-samples. The first sub-sample was used for physical properties with minimum structural disturbances. The second sub-sample was air-dried, crushed to pass through 2 mm mesh and stored in sealed plastic bags for subsequent analyses of chemical properties. The third sub-sample was carried to laboratory in ice box and immediately analysed for biological properties.

Grain and straw/stover were sampled randomly on five plants from each plot at harvest for N, P and Zn uptake analysis. Plant samples were washed with 0.01 N HCl followed by several washings with de-ionized water and oven dried at 65 ^{0}C to constant weight. Samples were ground in a Willey Laboratory Mill. Tissue N was determined by micro-Kjeldahl digestion, distillation and titration procedures (Bremner and Mulvaney 1982). Ground tissue was digested in a mixture of HNO_3:$HClO_4$ (3:1) and concentrations of P and Zn were determined by the ammonium molybdate (Olsen and Sommers 1982) and atomic absorption spectrophotometer (Perkin Elmer Analyst 200, USA), respectively.

Soil samples were analysed for pH (1:2 soil/water suspension) using a standard pH meter (Mettler Toledo, Model SevenEasy pH, GmbH, Switzerland), total N (by Kjeldahl method: digestion with concentrated H_2SO_4 in presence of K_2SO_4 and Zn dust at 360 ^{0}C in a Kjel Plus block digester, distillation in presence of excess NaOH and ammonia absorption in

boric acid; these steps were done by using Kjel Plus, Pelican Equipments, India. Then residual boric acid was titrated with standard 0.01 N H_2SO_4 and total P by digestion in HNO_3:$HClO_4$, 3:1, as described (Olsen and Sommers 1982). Available P (Bray's P) in soil was determined by stannous chloride blue color method (Bray and Kurtz 1945). Available Zn in soil was extracted by using DTPA as described by Liang and Karamanos (1993) followed by determination using atomic absorption spectrophotometer (Perkin Elmer Analyst 200, USA). Total organic C content in soil was determined by the dichromate oxidation method (Nelson and Sommers 1982). Soil aggregate analysis was done by wet sieving method (Camberdella and Elliott 1992). A 100 g soil sample (capillary-rewetted) was wet sieved by Yodder's apparatus through a series of sieves to obtain four size fractions: >2000 µm, 2000-250 µm, 250-53 µm and <53 µm. Aggregate fractions retained on each sieve transferred to glass beaker and oven dried at 65 ^{0}C for weight determination.

Soil biological properties

Several biological properties of soils from the six treatments were determined. Bacterial and fungal counts of soil were determined by serial dilution techniques (Subba Rao 1999). For analysis of microbial biomass-C (MBC), fungal biomass-C (FBC) and bacterial biomass-C (BBC) moist soil samples were pre-incubated at 25^{0}C for 36 h to attain basal respiration condition (Srivastava and Singh 1989). Microbial biomass C in pre-incubated soil samples (20 g dry weight equivalent) was determined by the chloroform fumigation-incubation technique (Jenkinson and Powlson 1976) using a K_c = 0.45 conversion factor (Witt et al. 2000). Fungal and bacterial biomass-C were determined using the method described by Hafeel et al. (2004) with some modifications. For FBC estimation, we used a mixture of fungal inhibitors amphotericin-B and captan to a final concentration of 0.5 and 2 mg g^{-1} soil, respectively. For

BBC, a mixture of bacterial inhibitors Rifampicin, Ampicillin, Chloramphenicol, Gentamycin and Streptomycin, each had a final concentration of 1 mg g^{-1} soil. These inhibitors were added to samples by mixing with talc powder (Bailey et al. 2002). Each soil sample was sub-divided into three equal sub-samples (20 g each). The control sub-sample received only talc @ 20 mg g^{-1} soil. Other two soil sub-samples received fungal and bacterial inhibitors, separately. The treatment mixtures were thoroughly mixed and incubated at 25^0C for 1 h. Then 1.0 ml of 0.4% glucose solution was added to each treatment tube, mixed thoroughly and inserted a glass test tube containing 5 ml of 0.5 N NaOH in each sample vial and stoppered with rubber bungs and re-incubated. Amount of CO_2 absorbed by NaOH was determined by titrating against standard 0.1 N H_2SO_4. Rest calculations were done as per the procedure followed for MBC determination. The MBC, FBC and BBC were expressed in terms of µg g^{-1} dry soil.

Earthworms' casts were counted in plots at the start of the 3rd year crop cycle by quadrate method. Plots were puddled, leveled and waited for the thin layer of water to disappear 7 days before transplanting of 7th crop, *Sali* rice, 2004. On the leveled plots, the earthworm casts appeared overnight and these casts were counted using 1 m^2 grid.

DNA extraction, polymerase chain reaction (PCR) and DGGE

Microbial DNA was extracted in freshly collected soil samples (500 mg) using the commercial FastDNA Spin Kit for soil (BIO101, Vista, CA). The soil DNA content ranged from 49.4 to 137.1 µg g^{-1} dry soil. Partial 16S rRNA gene fragments in the sample DNA were amplified using bacterial primers set described by Muyzer et al. (1993). Each amplification reaction (50 µl) contained 50 ng soil DNA, 1U *Taq* DNA polymerase, 200 µM dNTPs, in a 10 mM TrisHCl buffer (pH 8.0), 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100 and 32.5 p mol of each primer. Template DNA was omitted from negative control reaction. All

amplifications were performed at least twice for each DNA sample obtained from each replicate INM plot using a Hybaid Omnigene Thermocycler (Omnigene, The Netherlands) and reaction conditions were maintained as described by Muyzer et al. (1993). Amplified products were initially checked in agarose gel (1.5 % w v^{-1}).

Amplified products were loaded on 8% acrylamide gel using a denaturant gradient of 45-65% and run at 75 V, 60 ºC constant temperature for 18 h (Ingeny Phor mutation detection system (Ingeny International BV, The Netherlands). Gel was stained with ethiduim bromide (0.5 mg L^{-1}) and visualized under UV light on an Imago imaging system (Imago Scientific Instruments, USA). Number of bands in each profile was recorded. The relative intensity of a specific band was expressed as the ratio between the intensity of that band and the total intensity of DNA in a profile. Pair-wise similarity matrix among DGGE profiles based on numbers and relative abundances of DGGE bands using Dice correlation coefficient (Dice 1945) was determined. Hierarchical cluster analysis was performed using unweighted pair group method with arithmetic means (UPGMA) on similarity matrix to construct dendrogram to illustrate the relationship between bacterial community profiles of different nutrient management plots.

Statistical analysis

All statistical analyses were performed using SPSS v. 12.0 (SPSS Inc. Chicago, IL). We checked normality distribution among data generated from all replicated plots under six nutrient management treatments for each parameter using the Kolmogorov-Smirnov test and found normally distributed. For every parameter reported in this investigation, the six nutrient management treatments were analysed for differences among means ($P<0.05$) by performing

one-way analysis of variances (ANOVA) incorporating the Levene statistics to test the equality of group variances and the Least Significant Difference (LSD) test at $P<0.05$.

Results and Discussion

Grain yield and harvest index as influenced by bacterial bioinoculants based INM

Grain yields of *Sali* rice, *Ahu* rice and legume crop ranged from 2.4 to 3.8 Mg ha^{-1}, 1.6 to 3.7 Mg ha^{-1} and 0.15 to 1.3 Mg ha^{-1}, respectively across all nutrient management treatments (Table 2; $P<0.05$). Performance of the French bean crop in the 1st year crop cycle was poor i.e. 0.095–0.135 Mg (dry bean) ha^{-1} because of white mould disease in the crop.

The grain or pod yield of each crop under bacterial bioinoculants based INM plots was consistently higher (significant at $P<0.05$) compared to the yields under control plots and also increased marginally over that in compost alone or Urea:SSP:MOP plots (Table 2). In our earlier study, these bioinoculants (Azo and PSB) were found to be best among several strains tested in increasing grain yield of rice in field conditions compared to uninoculated control (Thakuria et al. 2004). Other workers reported 4.9 to 22% increase in yield of rice due to inoculation with *Azospirillum* compared to uninoculated control in field conditions (Lucy et al. 2004). Similarly, PSB strains were reported to vary in phosphate solubilization activity and stimulating growth of soyabean (Fernández et al. 2007). Reddy and Raju (2006) found that application of PSB with PR produced rice yields statistically *at par* with that produced by SSP application @ 30 kg ha^{-1}. The grain/pod yields for all nine crops under Urea:SSP:MOP or compost applied plots were *at par* to each other and this was expected as the soils of northeastern alluvial plains are highly responsive to externally added organic matter owing to

low organic C status (<8.0 g kg^{-1} soil) and very low NUE of applied inorganic fertilizers in these soils (Talukdar et al. 2004). During 3 years crop cycles, average increase in grain yield of *Sali* rice over control plots was 21.5%, 18.6% and 29.7%, of *Ahu* rice 33.8%, 33.3% and 46.2% and in pod yield of the legume 254.6%, 266.2% and 296.7% due to application of Urea:SSP:MOP, compost and bioinoculants based INM treatments, respectively (Table 2). This clearly indicated that response of legume crop (December-March) to applied nutrients was highest followed by *Ahu* (April–July) and *Sali* rice (August–November) in RLR rotation.

Harvest index (HI) of grain crops refers to the ratio of grain yield by total biomass yield (Rosielle and Frey 1975) and hence, HI can serve as a quality index for N management in cropping system. Harvest index of rice crops in Urea:SSP:MOP and urea added PSB alone INM plots was significantly (P<0.05) lower than HI in Azo/Rh plus PSB dual INM and Azo/Rh alone INM plots (Fig. 1). The higher quantity of straw production was responsible for the lower HI of rice crops in Urea:SSP:MOP and urea added PSB alone INM plots, which might be due to more availability of inorganic N through urea at early stages of crop growth. Aulakh et al. (2000) also reported that an excess supply of inorganic N at the early stages of crop growth encourages more vegetative growth. Overall, these results indicated that combine application of compost, Azo/Rh and PSB along with PR and MOP sustain higher yields under RLR rotation in acidic rice soils.

Nitrogenase activity as influenced by bacterial bioinculants based INM

Nitrogenase activity in roots of rice and pea (intact nodules) was determined in all nine crops except *Sali* rice, 2001 and French bean, 2001-02 (Fig. 2). In Azo/Rh alone INM and Azo/Rh plus PSB dual INM plots, nitrogenase activity was significantly (P<0.01) higher compared to that of other treatments. The ability to fix atmospheric N by the test Azo strain in rice roots

and also the synergistic effect of co-inoculation of Azo with PSB strain on N_2 fixation either *in-vitro* or in field conditions were previously reported (Thakuria 2006). Similar to observation by earlier workers (Roper and Ladha 1995), we also found that application of compost stimulated nitrogenase activity in both rice and pea roots. In contrast, inorganic fertilizer and urea included INM retarded nitrogenase activity but not significantly. Whether the stimulation or retardation in nitrogenase activity is associated with a corresponding stimulation/retardation of population of the N_2 fixing microorganisms can not be confirmed as we did not determine their population in soil. A very interesting observation was gradual increase in nitrogenase activities in roots of rice and pea under Azo/Rh alone INM or Azo/Rh plus PSB dual INM plots towards later crop cycles, which could be a result of either population build up of introduced bioinoculants in the rhizosphere or better soil environment for the N_2 fixers (Fig. 2). Although we observed persistence of these test strains in rice soil up to one year after inoculation (Boro et al. 2004), their population was not monitored yearly in this study. However, earlier research indicated that the counts of inoculated strains of *Azospirillum* and *Azotobacter* increased 2 to 3 folds in pearl millet rhizosphere when inoculation was continued for 3 years in fields with a corresponding increase in grain yield, nitrogenase activity and N assimilation (Wani et al. 1988).

Nitrogen uptake and balance

Nitrogen removed by grains of six crops plus straw of *Ahu* rice 2003 in different nutrient management plots differed significantly ($P<0.05$; Table 3) after completion of the 2nd year crop cycle. Nitrogen removed by six crops in control plots was the least (92 kg ha^{-1}). In compost and Urea:SSP:MOP plots, removal of N increased by 43.7% and 51% over control plots, respectively and in bacterial bioinoculants based INM plots removal of N further

increased by 7.2-14.7% and 12.7-20.5% over Urea:SSP:MOP and compost treated plots, respectively. Higher grain yields and N concentration in grain/pod and straw/stover of RLR rotation under bacterial bioinoculants based INM plots indicate more availability of N for crop uptake (Table 2 and 3). These results support a positive role for N_2 fixation by the test Azo and Rh strains in rice and pea crops, respectively.

Among three different bacterial bioinoculants based INM treatments, there was no statistically significant difference observed in the amount of N removed, despite of ~2 fold more N inputs added to soil in the urea added PSB alone INM treatment (Table 3). Surprisingly, in this treatment, the apparent N loss was approximately double the amount of apparent N loss observed in 0-15 cm soil depth in Azo/Rh plus PSB based INM or Azo/Rh alone INM plots (Table 3). But this higher apparent N loss could not be accounted for corresponding N removal value and apparent N gain in 15-30 cm soil depth suggesting N loss in relatively higher amount from soil of this treatment. This resulted in a low agronomic efficiency of applied N i.e. 8.5 kg grain kg^{-1} N in urea added PSB alone INM plots as against 14.1 kg grain kg^{-1} N in other two bioinoculants based INM plots. These results also suggest a positive role for nitrogen fixation in soils under Azo/Rh based INM treatments. The nitrogenase activity was also higher in these plots (Fig. 2).

The data on apparent N loss in 0-15 cm and apparent N gain in 15-30 cm soil depth under different treatments are in conformity with values of previous 14 reports that included 211 N balance values in rice based cropping system, out of which ninety-five percent values were in between –60 to +90 kg N $ha^{-1}crop^{-1}$ (Roger and Ladha 1992). The apparent loss of N at 0-15 cm depth not only supports differential N uptake by the six crops in different nutrient management treatments but also the movement of N from surface layer (0-15 cm) to the sub-surface layer (15-30 cm) as evident from positive soil total N balances at 15-30 cm depth. This data clearly indicated that application of N inputs in excess either as inorganic or organic

or in combination enhances loss of N from the system. Soils of the Brahmaputra basin are highly weathered and light in texture, and intense rain in the region seems to cause leaching of substantial amount of N from the surface layers particularly when the urea is a component of external nutrient inputs. Excessive use of N fertilizers is known to promote nitrate leaching (Aulakh et al. 2000; Ju et al. 2007). Under this situation, application of a bacterial bioinoculant based INM appeared to enhance N assimilation by crops, nitrogen fixation in soil and ability of the system to reduce N loss. The mechanisms of such beneficial effects of bacterial bioinoculants based INM approach need to be addressed in future research.

Phosphorus uptake and balance

Phosphorus removed by grains of six crops plus straw of *Ahu* rice 2003 in control plots was the least i.e. 22.8 kg ha^{-1} (Table 3). In compost alone and Urea:SSP:MOP plots, removal of P increased by 51.3% and 68.4% (significant at $P<0.05$) over control plots, respectively. Removal of P by six crops in bacterial bioinoculants based INM plots was significantly higher ($P<0.05$) compared to that in control or compost alone plots. Quantity of P removed in bacterial bioinoculants based INM plots was 7.6-12% higher over that in Urea:SSP:MOP plots (Table 3). These results indicated better P assimilation by the crops in bacterial bioinoculants based INM plots under RLR rotation in acidic soils.

After harvest of six crops, a net negative soil total P balance at 0-15 cm depth ranged from –26.4 to –61.3 kg P ha^{-1} (Table 3). The depletion of total P from the initial value at 0-15 cm soil depth can not be justified by the amount of total P removed by the six crops and suggests that two years of cultivation caused downward movement of P in soil as evident from a net positive soil total P balance of +28.1 to +88.1 kg P ha^{-1} at 15-30 cm soil depth irrespective of nutrient management (Table 3). Zhang et al. (2003) earlier also reported that

substantial quantity of molybdenum reactive P can move down from surface layers to deeper depth in paddy soils. We observed two distinct phenomena in relation to P management either through PSB with PR or SSP in acidic soil. The Azo/Rh plus PSB dual INM and PSB alone INM plots (both nutrient treatments received PSB with PR as source of P) showed least apparent loss of soil total P i.e. 1.6 and 0.8 kg ha^{-1}, respectively; whereas the Urea:SSP:MOP and Azo/Rh alone INM plots (both nutrient treatments received SSP as source of P) showed apparent gain in soil total P i.e. 11.4 and 6.0 kg ha^{-1}, respectively at 15-30 cm depth after harvest of six crops (Table 3). Therefore, application of readily soluble SSP in whole quantity as basal dose in light textured acidic soil under high rainfall areas might encourage translocation of substantial quantity of P to subsurface layer (15-30 cm soil depth). This leached down soluble form of P immediately bound by highly active Fe and Al oxides at sub-surface soil layer and thereby contributed a positive apparent soil total P balance. On the other hand, PR is insoluble in soil and with time slowly dissolves through various microbial mediated mechanisms (Kucey et al. 1989) and such available P fraction in soil was readily taken up by plants and thereby less chance of leaching losses to deeper depth and hence no positive apparent soil total P balance at 15-30 cm soil depth. The higher content of Bray's P in soil (significant at $P<0.05$) with corresponding higher quantities of P removal by crops under bacterial bioinoculants based INM plots also supported the positive role of the test PSB strain for better P assimilation by crops in RLR rotation (Table 3 and 5). The ability to solubilise tri-calcium phosphate *in-vitro* and enhancement of rice growth and yield under field condition by the test PSB strain was previously reported (Thakuria et al. 2004).

Depletion of DTPA extractable Zn in soil and Zn uptake by crop

Changes in soil DTPA extractable Zn in different nutrient management treatments after completion of 1st year crop cycle and also uptake and balance of Zn for the *Sali* rice 2002 are presented in the Table 4. The three bacterial bioinoculants based INM plots showed a significant (P<0.05) increase in soil DTPA extractable Zn content compared to that in control, Urea:SSP:MOP and compost plots (Table 4). A high correlation coefficient (r=0.83, P<0.01) between grain yields and DTPA extractable Zn contents in soil after 1-year RLR rotation indicated better Zn use efficiency in bacterial bioinoculants based INM plots compared to other treatment plots. Zinc removed by grain and straw of *Sali* rice 2002 was significantly (P<0.05) higher in bacterial bioinoculants based INM plots compared to the amount of Zn removed in control or compost plots. In control and compost alone plots, high apparent gain of DTPA extractable Zn in soil after harvest of *Sali* rice 2002 and lower quantity of Zn removed by that crop indicate the possibility of applied $ZnSO_4$ getting transformed to unavailable forms of Zn (clay-lattice bound, organic complexed, amorphous and crystalline sesquioxides-bound, Hazra et al. 1987) in soil of these plots. Such transformation of applied $ZnSO_4$ may also occur in INM plot soils but there is the possibility of solubilization of the bound fractions of Zn in the bioinoculants based INM plots. Hence, uptake of Zn by plant in these bioinoculants based INM plots was significantly (P<0.05) higher (Table 4). However, this need to be systematically investigated in future research. Raj (2002) also reported Zn-solubilization by a *Bacillus* sp in soil and improvement in grain yield and Zn uptake of rice. Therefore, improved Zn assimilation by the crops in bacterial bioinoculants based INM plots argues against a specific effect of the bioinoculants on N or P nutrition in cropping system.

Changes in total organic C content, aggregation, bacterial and fungal biomass, casting activities of earthworms and in composition of bacterial communities of soil

After completion of 2 years crop cycles, soil total organic C content depleted (ranged from 1.1 to 14.8%) at 0-15 cm depth and gained (ranged from 5.6 to 25.0%) at 15-30 cm soil depth across all nutrient management plots compared to initial total organic C content values in the respective soil depths (Table 5). Prior to this experiment, the field was lying fallow for 5 years. Therefore, this decline in total organic C at top layer might be associated with the cultivation induced factors (Dalal and Mayer 1986). Earlier, Naklang et al. (1999) observed depletion of soil organic C and labile C in 0-10 cm depth and gain of labile C in 20-40 cm depth following conversion of forest land to rainfed rice in light texture soil. However, the decline in soil total organic C was less in bacterial bioinoculants based INM plots and the reason could be better soil aggregation. Bacterial bioinoculants based INM plots showed significant (P<0.05) higher water-stable aggregation and mass of 2000-250 μm and 250-53 μm soil aggregates at 0-15 cm soil depth compared to that in control, Urea:SSP:MOP and compost alone plots after harvest of six crops in RLR rotation (Table 5). Mass of soil in aggregates class <53 μm increased significantly (P<0.05) in control, Urea:SSP:MOP and compost plots compared to that in bacterial bioinoculants based INM plots. The formation and stabilization of macro-aggregates (250-2000 μm) and micro-aggregates (53-250 μm) in these plots perhaps physically protected higher amount of particulate organic matter and hence, less chance of depletion of labile organic C from the surface layer (0-15 cm depth) to deeper soil depths. These macro- and micro-aggregates contain higher amounts of particulate and light fraction organic matters and that support higher rate of C and N mineralization in soils (Manna et al. 2005; Yan et al. 2007). Our results also indicate higher rate of C and N mineralization in bioinoculants based INM plots. However, laboratory incubation studies using soils both from the bioinoculants based INM and other treatment plots need to be carried out to generate data on C and N mineralization in future. Nevertheless, it is clear from the data that straw/stover incorporation with inorganic fertilizers or compost in light texture

alluvial soils might not be sufficient to counter balance the loss of soil organic C and deterioration of soil aggregation due to intensive cultivation under RLR rotation.

The Azo/Rh plus PSB dual INM and Azo/Rh alone INM and compost plots supported significant (P<0.05) higher BBC compared to that in control, Urea:SSP:MOP and urea added PSB alone INM plots (Fig. 3). In contrast, the control, Urea:SSP:MOP and urea added PSB alone INM plots supported significant (P<0.05) high FBC than the other nutrient management plots (Fig. 3). Population of bacteria and fungi determined in soils maintained a high correlation co-efficient (r = 0.87, P<0.01) with BBC and FBC, respectively in corresponding nutrient management plots (data not shown). The reflection of high MBC in control, Urea:SSP:MOP and urea added PSB alone INM plots was due to exceptional high contribution by FBC; FBC/BBC ratios were 3, 4.5 and 2.0, respectively in those plots. The high FBC/BBC ratios in Urea:SSP:MOP and urea added PSB alone INM plots might be due to lowering of soil pH, which stimulated fungal population significantly in those plots. After harvest of six crops, the maximum pH drop (0.24 units) observed in Urea:SSP:MOP plots followed by 0.20 units drop in urea added PSB alone INM plots (Table 5). The least pH drop (0.05 units) was observed in Azo/Rh plus PSB dual INM plots. Bååth and Anderson (2003) reported that fungal/bacterial ratio decreased significantly with increasing pH from about 9 at pH 3 to approximate 2 at pH 7.0. Although, reduction in pH in soils of control plots was not significant (Table 5), high FBC/BBC ratio in control plots could be a result of poor quality rice straw incorporation in soil (low nutrient content) and lower quantity of legume stover returned to the plots per RLR rotation. It has been reported earlier that low quality substrates (high C/N) favor fungi while high quality (low C/N) substrates favor bacteria in soil (Bossuyt et al. 2001). Thus we see that the bacterial bioinoculants based INM practice in RLR rotation ensure annual high return of quality legume stover along with rice straw and compost and associated biological N_2 fixation, consequently an improved status, uptake and balance of N

in soil (Table 3). As expected these factors caused an approximate balance of FBC/BBC ratio ranging from 1.1 to 1.3 in bacterial bioinoculants based INM plots that in turn helped to sustain a better nutrient mineralization process fueled by the labile C substrates in those plots. Higher N, P and Zn assimilation by crops coupled with high content of soil available N, P and Zn pools also supports the onset of a better mineralization process under bacterial bioinoculants based INM practice. Thus the fractionation of the MBC to FBC and BBC, and interpretation and use of their ratio in this study is justified as an index of better C and N mineralization process in soil. Because, MBC is a measure of biomass that is size not activity of microorganisms and therefore, use of MBC as a rapid indicator of C and N mineralization processes in soil may be misleading. Earlier, Witt et al. (1998) also reported that MBC measurement was a poor indicator of N mineralization–immobilization dynamics in soils. Overall, results of FBC/BBC ratios suggested that incorporation of legume and rice crop residues into soils along with external application of compost and bacterial bioinoculants under rice based rotation were useful in maintaining balance between C and N dynamics in soils. A balance between C and N dynamics in soils ensures more labile pools of soil organic C, and hence better mineralization processes of nutrients in soils.

After completion of 2 years crop cycles, earthworms' castings approximately doubled (significant at $P<0.05$) in Azo/Rh plus PSB dual INM and compost plots compared to that in Urea:SSP:MOP, SSP added Azo/Rh alone INM and urea added PSB alone INM plots (Table 5). Rice field earthworms are endogeics and preferentially feed on high quality soil organic matters. Endogeic earthworms preferably assimilate C from recently deposited fractions of soil organic matter, which is composed of more readily decomposable substances (Edwards and Arancon 2004). The bacterial bioinoculants based INM plots contained higher levels of readily decomposable substances like particulate organic matter due to higher percentage of macro- and micro-aggregates (Table 5). Again continuity of foods (for example more labile C

and N pools in the soil) through out the year attracted more earthworms in compost and bacterial biofertilizers based INM plots. However, addition of either urea or SSP reduced casting activities of earthworms in Azo/Rh alone INM, PSB alone INM and Urea:SSP:MOP plots. Though we do not know the exact cause for this reduction in casting activities of rice field earthworms, previous study reported that earthworm numbers and biomass were significantly less in inorganic fertilizer applied plots compared to manure-amended plots (Whalen et al. 1998), which needs further confirmation.

Hierarchical cluster analysis based on pair-wise similarity matrix of DGGE profiles showed that bacterial community (in terms of number of bands and their relative abundances) in control plots distinctly separated from the bacterial communities in other nutrient management plots (Fig. 4). The four DGGE bands (indicated by horizontal lines with square pointers in the lane 1, Fig. 4A) might represent succession of unique bacterial groups those are specifically abundant in nutrient poor soils. The variation in DGGE banding pattern in control plots compared to other nutrient management plots might be associated with preferential colonization of rice straw in soil by certain groups of bacteria under poor N availability condition, or otherwise decrease or elimination of abundant bacteria (for example DGGE bands indicated by the horizontal lines with arrow pointers in the lane 1, Fig. 4A) those prefer to colonize high quality soils, which needs further confirmation. Among nutrient input added plots, 2 distinct clusters at ≥82% similarity level were formed by the bacterial communities; one cluster represented by bacterial communities (>90% similarity) in Urea:SSP:MOP and urea added PSB alone INM plots and other one represented by the bacterial communities (>99% similarity) in Azo/Rh plus PSB dual INM and Azo/Rh alone INM plots (Fig. 4B). Except control plots, the differences of bacterial communities among nutrient input added plots were mainly due to change in relative band intensities, which indicates shift in abundances of dominant bacterial groups. Interestingly, C and N dynamics in

soils between Azo/Rh plus PSB dual INM and Azo/Rh alone INM plots were comparable, and soils of these two plots also harboured highly similar bacterial communities. Like-wise, C and N dynamics, bacterial community compositions in soils between Urea:SSP:MOP and urea added PSB alone INM plots were comparable. These findings implied that nutrient inputs such as legume and rice crops residues, bacterial bioinoculants, inorganic fertilizers added in different combinations to nutrient management plots modified community composition of soil bacteria through their direct influence on C and N dynamics.

Conclusions

Our study clearly demonstrated the multiple benefits of combined use of Azo/Rh with either PSB plus PR or SSP, MOP and compost in sustaining higher yields, better N, P and Zn assimilation by crops and improved soil quality under RLR rotation in acidic soil. These bacterial bioinoculants based INM formulations with incorporation of crop residues of RLR rotation in rainfed cropping system promoted more biological nitrogen fixation, better soil aggregation and earthworm activities and thereby regulated a better C and N dynamics in soils. The fungal/bacterial biomass C ratio in soil was found to be a better index of C and N dynamics for measurement of short-term changes in acidic soil under RLR rotation. Results of changes in bacterial community compositions in our experiment revealed the need of future study to investigate impacts of continuous use of bioinoculants on microbial community structure and its relation with soil functioning in cropping systems. We conclude that the INM formulation containing compost, *Azospirillum*/*Rhizobium* with either phosphate-solubilizing bacteria plus phosphate rock or SSP and MOP emerges as a superior nutrient management

option for rice-legume-rice rotation to counteract the factors associated with low productivity of the rainfed rice production systems in light texture acidic soils of the Brahmaputra basin.

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Figure legends

Fig. 1 Harvest index of rice crops influenced by different nutrient management treatments in rice-legume-rice rotation. Values that differ significantly (one-way ANOVA, $P<0.05$) within each cluster of dendrograms are followed by different letters. Azo is *Azospirillum*; Rh is

Rhizobium; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Fig. 2 Nitrogenase activity in roots of *Sali* rice (A), pea (B) and *Ahu* rice (C) influenced by different nutrient management treatments under rice-legume-rice rotation. Nitrogenase activity in roots of *Sali* rice and French bean of 1st year crop cycle were not determined. Each value on the line graph represents mean nitrogenase activity in roots of 12 plants from four replicated plots. Values that differ significantly (one-way ANOVA, $P<0.01$) on each line graph are followed by different letters. Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Fig. 3 Microbial biomass C (MBC), bacterial biomass C (BBC) and fungal biomass C (FBC) influenced by different nutrient management treatments determined after harvest of six crops in rice-legume-rice rotation. Values that differ significantly (one-way ANOVA, $P<0.05$) within each parameter are followed by different letters. Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Fig. 4 Denaturing gradient gel electrophoresis (DGGE) profiles of 16S rRNA gene fragments obtained by PCR amplification using bacterial primer sets (Muyzer et al. 1993) in soils of different nutrient management treatments. (A) an image of ethidium bromide stained DGGE gel and (B) hierarchical cluster plot based on similarity matrix of DGGE profiles. Joints of the branches of the dendrogram indicate the percentage similarity based on unweighted pair group method with arithmetic means (UPGMA). Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Figure1

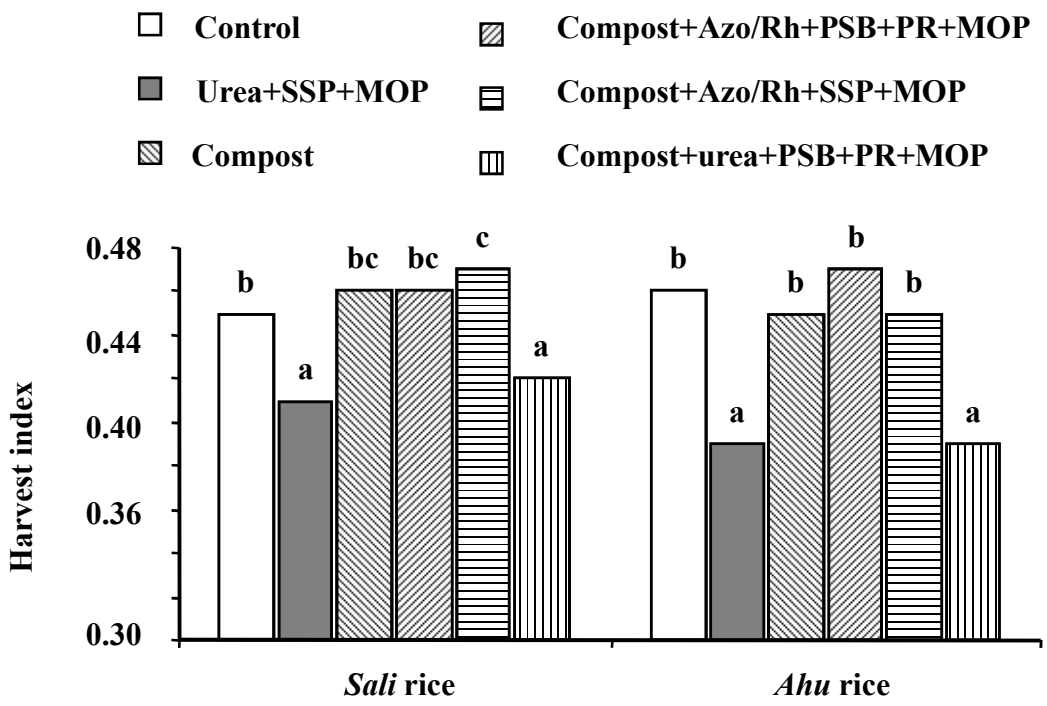
Control
Urea+SSP+MOP
Compost
Compost+Azo/Rh+PSB+PR+MOP
Compost+Azo/Rh+SSP+MOP
Compost+urea+PSB+PR+MOP
Harvest index
0.48
0.44
0.40
0.36
0.30
b
a
bc
bc
c
a
b
a
b
b
b
a
Sali rice
Ahu rice

Figure2

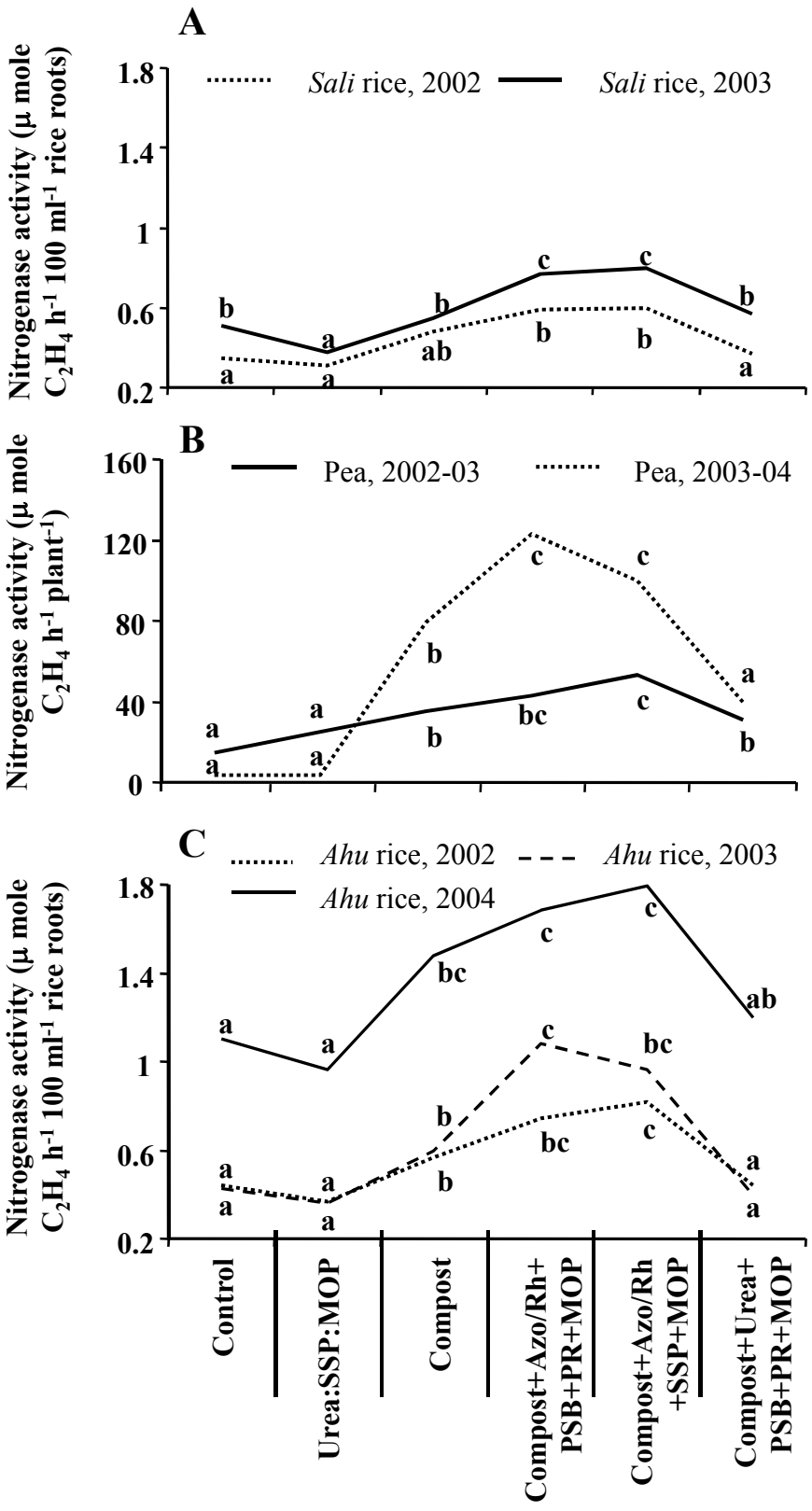
A
Nitrogenase activity (µ mole C_2H_4 h^{-1} 100 ml^{-1} rice roots)
Sali rice, 2002
Sali rice, 2003
1.8
1.4
1
0.6
0.2
B
Nitrogenase activity (µ mole C_2H_4 h^{-1} $plant^{-1}$)
Pea, 2002-03
Pea, 2003-04
160
120
80
40
0
C
Nitrogenase activity (µ mole C_2H_4 h^{-1} 100 ml^{-1} rice roots)
Ahu rice, 2002
Ahu rice, 2003
Ahu rice, 2004
1.8
1.4
1
0.6
0.2
Control
Urea:SSP:MOP
Compost
Compost+Azo/Rh+ PSB+PR+MOP
Compost+Azo/Rh +SSP+MOP
Compost+Urea+ PSB+PR+MOP

Figure3

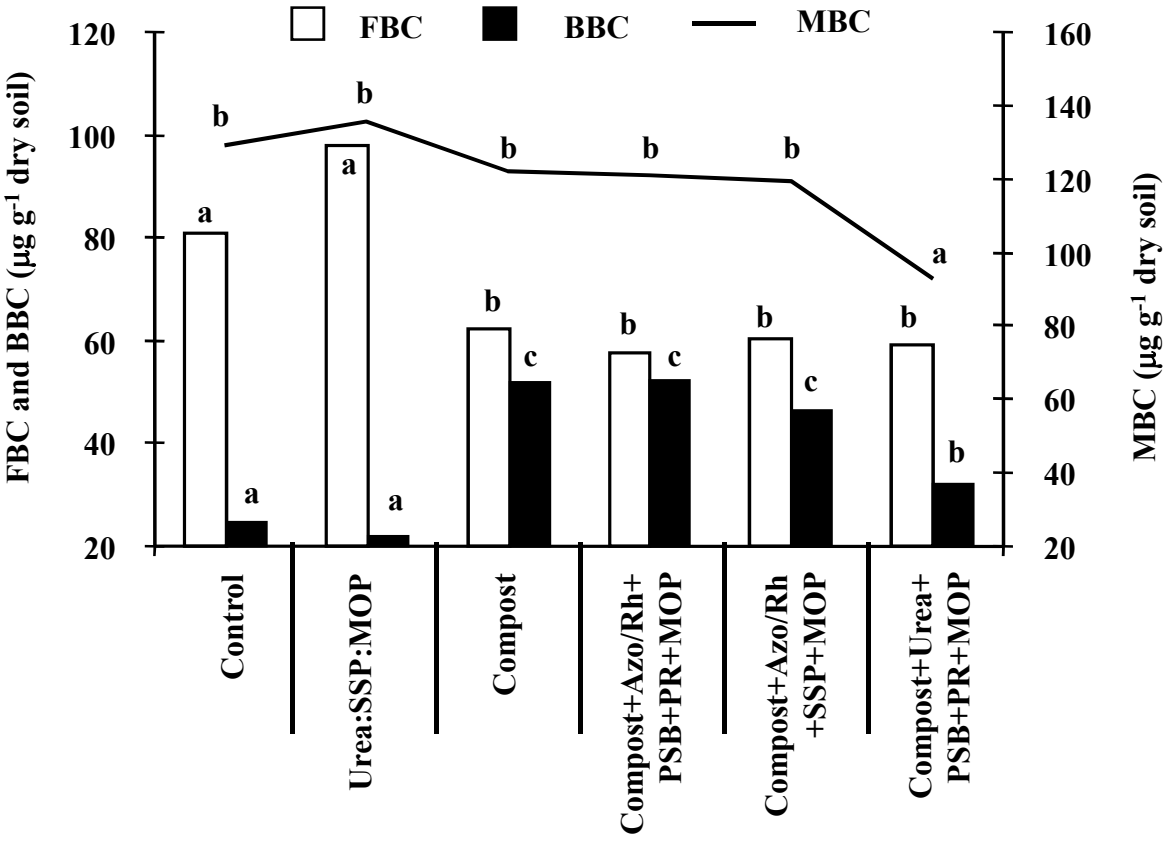
FBC
BBC
MBC
FBC and BBC (µg g-1 dry soil)
MBC (µg g-1 dry soil)
120
100
80
60
40
20
160
140
120
100
80
60
40
20
a
a
b
b
a
a
b
b
b
c
b
c
b
c
b
b
Control
Urea:SSP:MOP
Compost
Compost+Azo/Rh+ PSB+PR+MOP
Compost+Azo/Rh +SSP+MOP
Compost+Urea+ PSB+PR+MOP

Figure4

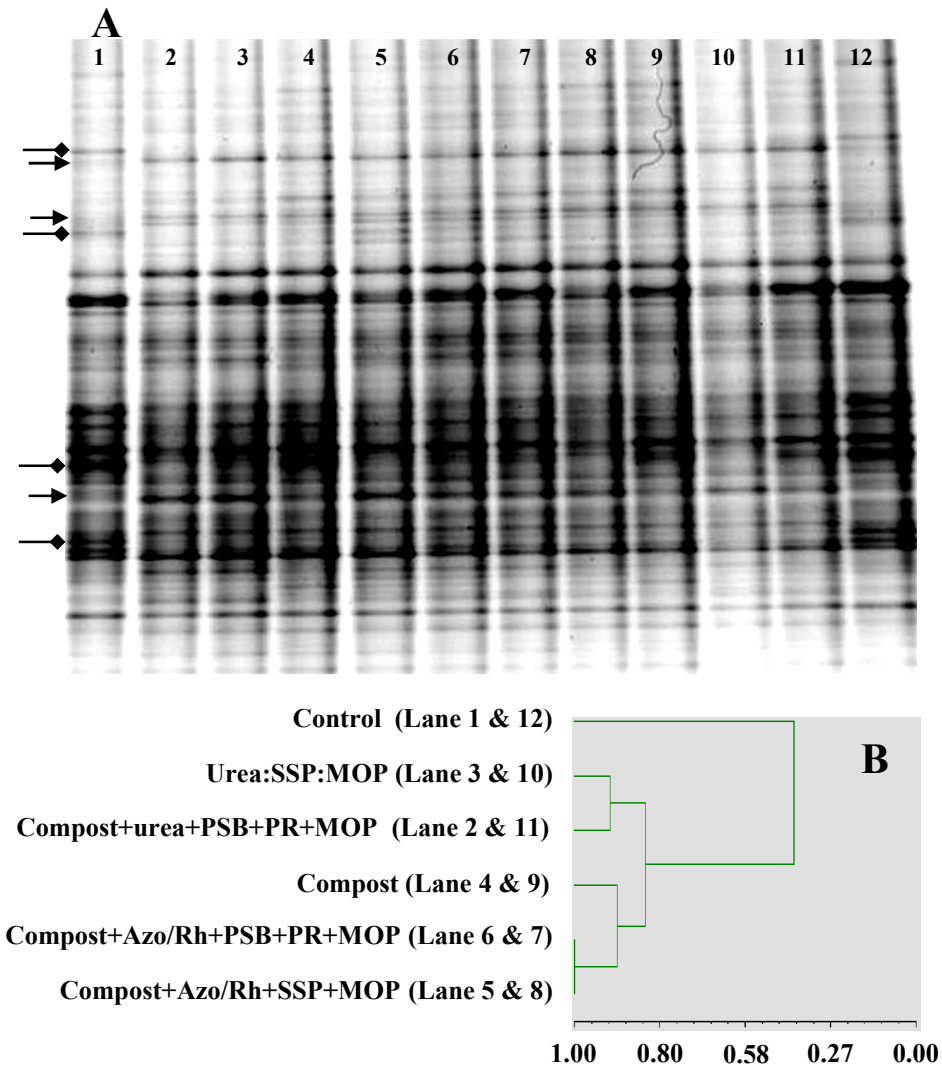
A
1
2
3
4
5
6
7
8
9
10
11
12
Control (Lane 1 & 12)
Urea:SSP:MOP (Lane 3 & 10)
Compost+urea+PSB+PR+MOP (Lane 2 & 11)
Compost (Lane 4 & 9)
Compost+Azo/Rh+PSB+PR+MOP (Lane 6 & 7)
Compost+Azo/Rh+SSP+MOP (Lane 5 & 8)
B
1.00
0.80
0.58
0.27
0.00

Table1

Table 1 Crop cycle and year, fertilizer application rate and form applied to nine crops in rice-legume-rice rotation during 2001-2004

Crop cycle & year	Season	Crop	Variety	Planting date	Harvesting date	Fertilizer rate[§]	Form of fertilizer[†]	Compost (Mg ha^{-1})
1st Year	*Kharif*	*Sali* rice	Ranjit	Aug. 14	Dec. 09	40:20:20	Urea:SSP:MOP	3
2001-02	*Rabi*	French bean	Contender	Feb. 05	Apr. 05	20:30/15:20	Urea:SSP/PR:MOP	2
	Summer	*Ahu* rice	Luit	Apr. 20	July 11	40:20/10:20	Urea:SSP/PR:MOP	3
2nd year	*Kharif*	*Sali* rice	Ranjit	July 28	Nov. 28	40:20/10:20 and 5	Urea:SSP/PR:MOP and $ZnSO_4$	3
2002-03								
	Rabi	Pea	Boneville	Nov. 15	Apr. 01	20:30/15:20	Urea:SSP/PR:MOP	2
	Summer	*Ahu* rice	Luit	Apr. 07	July 04	40:20/10:20	Urea:SSP/PR:MOP	3
3rd year	*Kharif*	*Sali* rice	Ranjit	Aug. 14	Dec. 10	40:20/10:20	Urea:SSP/PR:MOP	3
2003-04	*Rabi*	Pea	Azad	Nov. 19	Apr. 05	20:30/15:20	Urea:SSP/PR:MOP	2
	Summer	*Ahu* rice	Luit	Apr. 25	July 27	40:20/10:20	Urea:SSP/PR:MOP	3

[§]Applied rates were based on $N:P_2O_5:K_2O:ZnSO_4$ kg ha^{-1}, $ZnSO_4$ was applied only to the *Sali* rice crop of the 2nd year crop cycle.
[†]PR was applied @ ½ of the recommended rate of SSP to all the PSB applied plots instead of SSP. PR was not applied to the *Sali* rice of 1st year crop cycle. PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Table 2 Effect of bacterial bioinoculants based integrated nutrient management on grain yields of nine crops in rice-legume-rice rotation[§]

Crop / Nutrient management	1st year crop cycle (2001-2002)				2nd year crop cycle (2002-2003)				3rd year crop cycle (2003-2004)			
	Sali rice	French bean	*Ahu* rice	**Total**	*Sali* rice	Pea	*Ahu* rice	**Total**	*Sali* rice	Pea	*Ahu* rice	**Total**
	Grain yield (Mg ha^{-1})											
Control	2.35a	0.019a	1.60a	**3.97a**	2.81a	0.34a	1.59a	**4.74a**	2.39a	0.15a	2.35a	**4.89a**
Urea:SSP:MOP	2.53ab	0.095b	2.08ab	**4.71b**	3.74b	0.50ab	2.19b	**6.43b**	2.67ab	1.21b	2.44a	**6.32b**
Compost	2.64b	0.094b	1.92ab	**4.65b**	3.51b	0.61ab	2.33bc	**6.45b**	2.78b	1.16b	2.53a	**6.46b**
Compost+Azo/Rh +PSB+PR+MOP	2.96c	0.098b	2.33bc	**5.39c**	3.75b	0.81b	2.34bc	**6.90bc**	2.82b	1.29b	3.21b	**7.33c**
Compost+Azo/Rh +SSP+MOP	2.74bc	0.135c	2.08ab	**4.96bc**	3.70b	0.77ab	2.56cd	**7.03c**	2.86b	1.30b	3.08b	**7.24c**
Compost+urea+ PSB+PR+MOP	2.64b	0.110bc	2.59c	**5.34c**	3.76b	0.52ab	2.79d	**7.07c**	2.83b	1.03b	3.67c	**7.53c**

[§]Each value represents mean yield from four replicated plots and values that differ significantly (one-way ANOVA, $P<0.05$) within each column are followed by different letters. Grain yield was reported at 120 g kg^{-1} moisture content. Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Table3

Table 3 Removal of N and P by crops and apparent N and P balances in soil at 0-15 cm and 15-30 cm depth after harvest of six crops during 2 year crop cycle in rice-legume-rice rotation[§]

Nutrient management	Soil total N (kg ha^{-1})		N (kg ha^{-1}) removed by the six crops	Apparent loss/ gain of soil total N (kg ha^{-1}) at 0-15 and 15-30 cm depth	Soil total P (kg ha^{-1})		P (kg ha^{-1}) removed by the six crops	Apparent loss/ gain of soil total P (kg ha^{-1}) at 0-15 and 15-30 cm depth
	At beginning of *Sali* rice, 2001	At harvest of *Ahu* rice, 2003			At beginning of *Sali* rice, 2001	At harvest of *Ahu* rice, 2003		
Control	2381.1a	2189.2	92.2a	-99.7	460.1a	398.8	22.8a	-38.5
	1233.5a	**1338.0**		**+4.8**	**220.0a**	**248.1**		**-10.4**
Urea:SSP:MOP	2414.7a	2259.7	139.2b	-215.8	450.0a	391.6	38.4bc	-76.7
	1253.5a	**1525.3**		**+56.0**	**224.0a**	**312.1**		**+11.4**
Compost	2387.5a	2305.4	132.5b	-183.4	459.0a	408.1	34.5b	-52.8
	1243.0a	**1603.0**		**+177.0**	**230.0a**	**275.2**		**-7.6**
Compost+Azo/Rh +PSB+PR+MOP	2391.8a	2325.1	151.2c	-159.3	468.6a	442.2	43.0c	-63.5
	1240.5a	**1653.4**		**+253.6**	**226.0a**	**287.9**		**-1.6**
Compost+Azo/Rh +SSP+MOP	2393.9a	2320.2	149.3c	-168.2	479.7a	450.6	42.7c	-79.6
	1245.5a	**1679.8**		**+236.1**	**228.0a**	**313.6**		**+6.0**
Compost+urea+ PSB+PR+MOP	2427.4a	2325.1	159.6c	-376.5	469.7a	439.0	41.3c	-69.5
	1260.5a	**1779.7**		**+142.7**	**221.0a**	**289.7**		**-0.8**

[§]Values in plain and bold fonts depict 0-15 cm and 15-30 cm soil depth, respectively and also values with – and + signs indicate loss and gain, respectively. Values that differ significantly (one-way ANOVA, $P<0.05$) within each column are followed by different letters.
Variation in soil total N content at depth 0-15 cm or 15-30 cm throughout the experimental field at the beginning of *Sali* rice 2001 was < 2.1%.
Variation in soil total P content at depth 0-15 cm or 15-30 cm throughout the experimental field at the beginning of *Sali* rice 2001 was < 6.4%.
Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Table4

Table 4 Changes in DTPA extractable Zn in soil at 0-15 cm depth after completion of first year rice-legume-rice rotation and apparent DTPA extractable Zn balance in soil during growth of *Sali* rice 2002*

Nutrient management	DTPA extractable Zn (kg ha^{-1})		Changes in DTPA-Zn (kg ha^{-1}) after 1st year crop cycle	Zn (kg ha^{-1}) added to *Sali* rice, 2002	Zn (kg ha^{-1}) removed by *Sali* rice, 2002	DTPA extractable Zn (kg ha^{-1}) at harvest of *Sali* rice, 2002	Apparent loss/gain of DTPA-Zn (kg ha^{-1}) at 0-15 cm depth †
	At transplant of *Sali* rice, 2001§	At harvest of *Ahu* rice, 2002					
	(a)	**(b)**	**(a-b)**	**(c)**	**(e)**	**(d)**	**(b+c) – (d+e)**
Control	1.40a	0.98a	-0.42	1.14	0.28a	0.96a	+0.88
Urea:SSP:MOP	1.33a	1.12ab	-0.11	1.14	0.45c	1.23ab	+0.55
Compost	1.47a	1.32bc	-0.15	1.14	0.37b	1.39b	+0.70
Compost+Azo/Rh +PSB+PR+MOP	1.31a	1.62cd	+0.31	1.14	0.50c	2.24c	+0.02
Compost+Azo/Rh +SSP+MOP	1.52a	1.74d	+0.22	1.14	0.44bc	2.29cd	+0.15
Compost+urea+ PSB+PR+MOP	1.49a	1.53cd	+0.04	1.14	0.49c	2.66d	-0.48

§ Variation of DTPA extractable Zn contents at soil depth 0-15 cm throughout the experimental field at transplant of *Sali* rice 2001 was < 8.7%;
† Values with – and + signs indicate loss and gain, respectively.
*Values that differ significantly (one-way ANOVA, P<0.05) within each column are followed by different letters.
Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.

Table 5 Effects of bacterial bioinoculants based integrated nutrient management on soil pH, organic C, water stable aggregation, aggregates size distribution, number of casts of earthworms and Bray's P content in soil after growth of six crops in rice-legume-rice rotation[§]

Nutrient management	Unit change in pH at 0-15 cm soil depth [δ]	% change in organic C [†]		Water stable aggregation of soil (%)	Mass of soil in aggregate class [¶] (%)			No. of earthworms' casts m^{-2}	Bray's P (kg ha^{-1}) at harvest of *Ahu* rice 2003
		At 0-15 cm depth	At 15-30 cm depth		2.0 mm - 0.25 mm	0.25 mm - 0.53 mm	< 0.53 mm		
Control	-0.08	-14.8	+5.6	70.0a	26.60a	27.23a	34.22c	158.3a	17.1a
Urea:SSP:MOP	-0.21	-11.4	+5.6	73.7ab	27.12a	29.51a	30.36bc	455.6b	20.8b
Compost	-0.10	-11.4	+13.9	76.7b	29.07ab	31.82a	22.37b	847.2c	20.3b
Compost+Azo/Rh +PSB+PR+MOP	-0.05	-4.5	+25.0	84.0c	33.33b	40.06b	10.10a	738.9c	29.6d
Compost+Azo/Rh +SSP+MOP	-0.09	-1.1	+33.3	87.3c	34.50b	41.96b	9.98a	452.8b	23.5c
Compost+urea+ PSB+PR+MOP	-0.18	-6.8	+22.2	82.5c	33.62b	42.55b	9.01a	416.7b	23.2c

[§]Values that differ significantly (One-way ANOVA, $P<0.05$) within each column are followed by different letters.

[δ] Values with - sign indicates unit drop from the initial soil pH 4.80.

[†] Values with - and + signs indicate loss and gain, respectively over the initial total organic C contents 8.8 and 6.7 g kg^{-1} soil at 0-15 and 15-30 cm depth, respectively.

[¶] Values for mass of soil in aggregates class > 2.0 mm are not shown in the table as there was no statistical significance different among treatments.

Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is muriate of potash.