

University of Warwick institutional repository: <http://go.warwick.ac.uk/wrap>

This paper is made available online in accordance with publisher policies. Please scroll down to view the document itself. Please refer to the repository record for this item and our policy information available from the repository home page for further information.

To see the final version of this paper please visit the publisher's website. Access to the published version may require a subscription.

Author(s): Dwipendra Thakuria, Narayan C. Talukdar, Chandan Goswami, Samarendra Hazarika, Mohan C. Kalita<sup>4</sup> and Gary D. Bending

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

Year of publication: 2009

Link to published version: <http://dx.doi.org/10.1007/s00374-008-0320-4>

Publisher statement: The original publication is available at [www.springerlink.com](http://www.springerlink.com)

Editorial Manager(tm) for Biology and Fertility of Soils  
Manuscript Draft

Manuscript Number: BFSO-D-08-00029R2

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

Corresponding Author: Dr. Dwipendra Thakuria, Ph.D.

Corresponding Author's Institution: University College Dublin

First Author: Dwipendra Thakuria, PhD

Order of Authors: Dwipendra Thakuria, PhD; Narayan C Talukdar, PhD; Chandan Goswami, PhD; Samarendra Hazarika, PhD; Mohan C Kalita, PhD; Gary D Bending, PhD

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<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 40:20:20 kg ha<sup>-1</sup> for rice and 20:30:20 kg ha<sup>-1</sup> 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  $\mu$ m and 250-53  $\mu$ 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.

Suggested Reviewers: Paolo Nannipierri

paolo.nannipieri@unifi.it

Prof. Nannipierri reviewed earlier versions of this manuscript

Kazuyuki Inubushi

inubushi@faculty.chiba-u.jp

Prof. Inubushi reviewed first version of this manuscript

## Author's response to reviewers' comments\_R2

**Ref.: Ms. No. BFSO-D-08-00029R1**

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.....cropping system on grain yield, nutrient uptake, nitrogen fixation, and chemical, physical and biological properties 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 titrated with standard...HClO<sub>4</sub>, 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<sub>2</sub>SO<sub>4</sub> and total P by digestion in HNO<sub>3</sub>:HClO<sub>4</sub>, 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<sup>-1</sup> 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 µm and 250-53 µm soil aggregates at 0-15 cm.....(Table 5. Mass of soil in aggregates class <53 µm 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 1<sup>st</sup> 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**

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 Type of contribution: Full length (Original Research)  
2 Date of preparation: January 24, 2008  
3 Date of revised submission: July 4, 2008  
4 Number of text pages: 34  
5 Number of tables: 5  
6 Number of figures: 4

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

12 Name of Authors: **Dwipendra Thakuria<sup>1\*</sup> · Narayan C. Talukdar<sup>2\*</sup> · Chandan Goswami<sup>2</sup> · Samarendra Hazarika<sup>3</sup> · Mohan C. Kalita<sup>4</sup> · Gary D. Bending<sup>5</sup>**

16 Affiliation: <sup>1</sup>Krishi Vigyan Kendra, Assam Agricultural University, Napaam, Tezpur 784 028, Assam, India

<sup>2</sup> Soil Microbiology Laboratory, Department of Soil Science, Assam Agricultural University, Jorhat 785013, Assam, India

<sup>3</sup>Department of Agricultural Engineering, Assam Agricultural University, Jorhat 785013, Assam, India

<sup>4</sup>Department of Biotechnology, Gauhati University, Guwahati, Assam, India

<sup>5</sup>Nutrient and Pesticide Dynamic Programme, Warwick HRI, Warwick, CV35 9EF, UK

32 \* Corresponding authors: <sup>1</sup> UCD School of Biology and Environmental Science, Science Education and Research Centre (West), University College Dublin, Dublin 4, Ireland  
35 Email: thakuria.dwipendra@yahoo.co.in;  
36 Tel.: 00353-1-7162343, Fax: 00353-1-7161153

<sup>2</sup> Microbial Resources Division  
Institute of Bioresources and Sustainable Development, Department of Biotechnology, GOI, Imphal, Manipur, India  
42 Email: nctalukdar@yahoo.com;  
43 Tel: 0091-385-2446122, Fax: 0091-385-2446120



**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<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 40:20:20 kg ha<sup>-1</sup> for rice and 20:30:20 kg ha<sup>-1</sup> 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  $\mu$ m and 250-53  $\mu$ 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

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

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

## 81 **Introduction**

83 To increase crop productivity under rainfed rice cropping systems in sustainable manner,  
84 efficient nutrient management approach needs to be developed keeping in view the factors of  
85 low productivity inherent in the systems. In northeastern alluvial plains of India, factors of  
86 low productivity of rice are (a) the nutrient content in soil and their use efficiency (NUE) is  
87 low, for example highly weathered light texture alluvium soils of Brahmaputra basin are  
88 prone to intense leaching losses of applied nitrogenous fertilizers coupled with high (> 81%)  
89 fixation rate of applied phosphatic fertilizers due to high activities of Fe and Al oxides, and  
90 Zn deficiency; (b) lack of site-specific efficient nutrient management approach; (c) low soil  
91 organic carbon (SOC) content (<8.0 g kg<sup>-1</sup>); (d) uneven distribution of rainfall throughout  
92 crop growing periods; (e) no or little inorganic fertilizers (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O) use @ 13 kg ha<sup>-1</sup> in  
93 the northeastern region of India and (f) poor economic condition of farmers (Tewari et al.

1  
2  
3  
4 94 [1969](#); Talukdar and Chakravarty [1988](#); Khan et al. [2004](#); Talukdar et al. [2004](#)). Under such  
5  
6 95 conditions, increasing cropping intensity from double to triple in a year, without affecting soil  
7  
8 96 quality is a major challenging task. This demands that several different aspects including  
9  
10 97 cultivation of right crop in rotation with rice, recycling of crop residues and efficient nutrient  
11  
12 98 management approach inclusive of different sources of nutrients are addressed through  
13  
14 99 systematic research. Combined application of inorganic fertilizers with blue-green algae or  
15  
16  
17 100 green manure or farmyard manure with or without crop residue incorporation is known to  
18  
19 101 improve NUE and higher yields in rice-based cropping systems (Regmi et al. [2002](#);  
20  
21 102 Yadvinder-Singh et al. [2004](#); Reddy and Raju [2006](#); Pampolino et al. [2007](#)).

22  
23  
24 103 Inclusion of legume crop in rotation is an important aspect of N and C management in  
25  
26 104 fragile soils (Ladha and Reddy [2003](#)) and also an opportunity to meet the perpetuated deficit  
27  
28 105 in per capita availability of pulses in India (Prasad and Nagarajan [2004](#)). Besides being a N  
29  
30 106 rich grain, legume crop can also serve the role of green manure in the triple cropped rice  
31  
32 107 systems by contributing N and biomass to the soil (George et al. [1994](#); Dobermann and White  
33  
34 108 [1999](#); Yadav [2003](#)). Application of inorganic N fertilizer at higher rate to boost crop  
35  
36 109 productivity in acidic soils under rainfed rice systems is not a profitable N management  
37  
38 110 approach due to very low N use efficiency. A recent study has indicated that use of inorganic  
39  
40 111 N fertilizer at rate exceeding grain N removal caused a net decline in soil C despite  
41  
42 112 increasingly massive residue C incorporation (Khan et al. [2007](#)). Therefore, we presumed that  
43  
44 113 any N management strategy that involves higher rate of inorganic N fertilizer application  
45  
46 114 together with crop residue incorporation into soil to boost high yields from rainfed rice  
47  
48 115 systems would be a suicidal approach. In this context, concept of integrated nutrient  
49  
50 116 management (INM) might be fruitful in increasing grain yields in triple cropped rice-legume-  
51  
52 117 rice (RLR) rotation and also in sustaining soil productivity and overall environmental quality  
53  
54 118 (DeDutta [1989](#); Yadav [2003](#); Pampolino et al. [2007](#)). Use of bacterial bioinoculants such as  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 119 azospirilla or rhizobia with phosphate solubilizing bacteria and phosphate rock (a slow release  
5  
6 120 mineral P source) with compost and crop residue incorporation might increase NUE of major  
7  
8 121 limiting nutrients N, P and Zn including better management of soil C under RLR rotation in  
9  
10 122 acidic soils (Ladha and Reddy 2003; Somado et al. 2003; Choudhury and Kennedy 2004).  
11  
12 123 Many previous studies confirmed the benefits of single or dual inoculation of phosphate  
13  
14 124 solubilizing bacteria with azospirilla or rhizobia to cereals and legumes (Jeyabal and  
15  
16 125 Kuppaswamy 2001; Johri et al. 2003; Somado et al. 2003; Choudhury and Kennedy 2004;  
17  
18 126 Lucy et al. 2004; Reddy and Raju 2006). However, data on performance (in terms of grain  
19  
20 127 yield, nutrient balance and soil quality) of single or dual inoculation of these beneficial  
21  
22 128 microorganisms as components of INM in acidic rice soils under RLR rotation and residue  
23  
24 129 incorporation over several seasons are limited.

25  
26  
27  
28  
29 130 The objective of this study was to determine performance of bacterial bioinoculants  
30  
31 131 (*Azospirillum*, *Rhizobium* and phosphate solubilizing bacteria) based INM treatments against  
32  
33 132 the existing farmers' nutrient management practices (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 40:20:20 kg ha<sup>-1</sup> for rice  
34  
35 133 and 20:30:20 kg ha<sup>-1</sup> for legume) for RLR rotation in acidic alluvial soils of northeastern  
36  
37 134 plains of India in order to achieve higher productivity and soil sustainability. The performance  
38  
39 135 comparison was done in terms of grain yields, uptake and balance of N, P and Zn and changes  
40  
41 136 in organic C, aggregation, bacterial and fungal biomass C, casting activities of earthworms in  
42  
43 137 soil. We also assessed the impacts of continuous application of bacterial bioinoculants based  
44  
45 138 INM on composition of bacterial communities of soils under different nutrient managements  
46  
47  
48 139 in RLR rotation.

49  
50  
51 140

52  
53 141

## 54 55 142 **Materials and methods**

56  
57 143  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 144 Experimental location and climate  
5

6 145  
7

8 146 A field experiment was set-up at the experimental farm of Assam Agricultural University  
9  
10 147 (24<sup>0</sup>46' N, 94<sup>0</sup>13' E and 87 m above mean sea level) located in Assam, India. The field was  
11  
12 148 not cultivated in the last 5 years prior to this experiment. Climate of the region is typic sub-  
13  
14 149 tropical humid and receives mean annual rainfall 1931 mm and average rainy days 157 per  
15  
16 150 annum. Total bright sunshine hour (BSSH) is 2129 hours against maximum possible BSSH of  
17  
18 151 4432 hours per year. Mean relative humidity is 79%. During experimental years 2001–2004,  
19  
20 152 the mean maximum and minimum temperatures recorded during *Sali* rice (*Kharif*, August-  
21  
22 153 November) seasons were 30.6 and 21.7 °C, legume (*Rabi*, December-March) seasons were  
23  
24 154 25.0 and 11.0 °C and *Ahu* rice (*Summer*, April-July) were 30.9 and 21.6 °C, respectively. The  
25  
26 155 length of crop growing period (LGP) is >210 days in a year in this agro-ecological zone.  
27  
28  
29  
30

31 156  
32

33 157 Plot layout, soil characteristics, crops and treatments  
34  
35

36 158  
37

38 159 The Experimental field was divided into six blocks. Each block represented one nutrient  
39  
40 160 treatment (see below for treatments detail) and within each block, four plots were the  
41  
42 161 replicates each with an area of 4 x 5 m<sup>2</sup>. Each block was laterally isolated by polythene sheets  
43  
44 162 embedded into the soil to a depth 30 cm. The experiment was arranged as completely  
45  
46 163 randomized block design. An uniformity trial of soil fertility on the experimental field was  
47  
48 164 carried out before the start of the RLR rotation crops by growing high yielding *Ahu* rice  
49  
50 165 (*Summer* rice) variety 'Luit' in close spacing (10 cm x 10 cm, between rows x plants).  
51  
52  
53

54 166 The initial soil characteristics of the experimental field were determined after  
55  
56 167 completion of the uniformity trial. The sandy loam inceptisol (Oxyaquic Dystrocrept) had the  
57  
58 168 following properties: sand 55%, silt 30%, clay 15%, bulk density 1.36 Mg m<sup>-3</sup>, pH (1:2,  
59  
60  
61  
62  
63  
64  
65

169 soil:water) 4.80, total organic C 8.8 g kg<sup>-1</sup> soil, total N 1.07 g kg<sup>-1</sup> soil, total P 210 mg kg<sup>-1</sup>  
170 soil, diethylenetriaminepentaacetate (DTPA) extractable Zn 0.62 mg kg<sup>-1</sup> soil, cation  
171 exchange capacity 3.17 cmol kg<sup>-1</sup> soil, base saturation 65.5% and water holding capacity 372  
172 g kg<sup>-1</sup> soil.

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

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

1  
2  
3  
4 194 amounts of the recommended quantities of SSP (inorganic P) and MOP (inorganic K) were  
5  
6 195 applied in the puddled plots as basal application one day before transplanting of rice seedlings  
7  
8 196 and the remaining half quantity of urea was top dressed on standing rice crop after 30 days of  
9  
10 197 transplantation (DAT). Urea, SSP and MOP were applied as basal dose to the legume crops in  
11  
12 198 rows 2 days before seeding. Compost was applied to respective treatment plots 10 days before  
13  
14 199 transplanting in case of rice or at the time of seeding in case of pea. Crop-wise fertilizer dose  
15  
16 200 applied to nine crops during 3 years crop cycles are presented in Table 1.

17  
18  
19  
20 201 Dry compost (particle size <1 mm) in 500 g packets were double sterilized (at 121 °C  
21  
22 202 under 0.11 MPa for 15 min twice at 36 h interval) to ensure complete sterilization and used as  
23  
24 203 carrier material for bioinoculants Azo, Rh and PSB. Known quantity of broth culture of each  
25  
26 204 bioinoculant was mixed separately with sterilized compost as described by Thakuria et al.  
27  
28 205 [2004](#). The number of cells of Azo, PSB and Rh were  $3.5 \times 10^9$ ,  $3.3 \times 10^8$  and  $2.9 \times 10^9$  *cfu g*<sup>-1</sup>  
29  
30 206 compost, respectively. The compost-based Azo, Rh and PSB bioinoculants were applied (@ 4  
31  
32 207 kg ha<sup>-1</sup>) to rice seedlings by root-dip technique. The required quantity of bioinoculants was  
33  
34 208 made into slurry and the rice seedlings of respective treatments were dipped for 3 h prior to  
35  
36 209 transplanting. By this technique the bioinoculants were adhered to the seedling roots. After  
37  
38 210 root-dip treatment of rice seedlings, the average population of Azo and PSB determined on  
39  
40 211 inoculated rice seedling roots were  $8.3 \times 10^7$  *cfu* on rojo congo agar (Cáceres [1982](#)) and  $7.8 \times$   
41  
42 212  $10^6$  on Pikovskaya's agar (Sundara Rao and Sinha [1963](#)), respectively. Pea seeds were coated  
43  
44 213 with Rh and average *cfu* per coated seed determined on yeast extract mannitol agar (Subba  
45  
46 214 Rao [1999](#)) was  $6.9 \times 10^6$ . French bean seeds were coated with *Rhizobium phaseoli* (FB-9-2)  
47  
48 215 and *cfu* on seeds were not quantified.

49  
50  
51  
52  
53 216

54  
55  
56 217 Crop harvesting and residue recycling

57  
58  
59 218

1  
2  
3  
4 219 Both *Sali* and *Ahu* rice were harvested at physiological maturity stage. Pods of french bean  
5  
6 220 and pea crops were picked up thrice in sequence. French bean pods were harvested as green  
7  
8 221 vegetable, whereas the pea was harvested as mature pods. *Ahu* rice straw and legume stover  
9  
10 222 were harvested just at level to the soil surface. The fresh rice straw and legume stover from  
11  
12 223 each plot were weighed and a uniform sample of 2 kg (rice) and 1 kg (legume) withdrawn,  
13  
14 224 oven-dried at 65 °C to constant weight and weighed. Oven-dry weight of the sample was used  
15  
16 225 to convert the fresh straw and stover weight on oven-dry basis. The remaining portion of the  
17  
18 226 straw and stover was again immediately incorporated into the soil of respective plots. In case  
19  
20 227 of *Sali* rice of 2<sup>nd</sup> and 3<sup>rd</sup> year rotations, panicles were harvested for grain yield and the straw  
21  
22 228 yield was estimated by cutting only 10 hills at ground level in uniform pattern from each plot.  
23  
24  
25  
26  
27  
28

29 230 Nutrient balance in soil after harvest of six crops (2 years rotation)

30  
31 231

32  
33 232 The depth-wise (at 0-15 cm and 15-30 cm) nutrient balance sheets in soil were calculated at  
34  
35 233 the end of second year rotation. Straw and stover of rice and legume crops were incorporated  
36  
37 234 into soil and hence, nutrient removed by six crops referred to the grain nutrient uptake by six  
38  
39 235 crops plus nutrient uptake by straw of the *Ahu* rice 2003 i.e. the sixth crop. In this study, the  
40  
41 236 possibilities of inputs error through rice seedlings, legume seeds and also from rainfall to  
42  
43 237 nutrient treatment plots were negligible for nutrient balance calculation, because each  
44  
45 238 treatment plot received equal numbers of rice seedlings (same age) and legume seeds as  
46  
47 239 planting materials and also equal amount of rainfall. During dry spell in each *Ahu* rice season,  
48  
49 240 one life saving irrigation was given in equal volume from the same irrigation source to all  
50  
51 241 treatment plots and it was assumed that any nutrient added to the plots through irrigation  
52  
53 242 water was in equal amount and did not affect nutrient balance results. There was no flood on  
54  
55 243 the plots during the *Sali* (monsoon) seasons of experimentation period and thus assumed to  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



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

250 N or P balance =  $\sum$  (N or P from compost & inorganic fertilizers) – plant N or P (uptake in  
251 grain & straw or stover)

254 Determination of nitrogenase activity by acetylene reduction assay

255  
256 Closed acetylene reduction assay (ARA) can accurately indicate relative differences in  
257 nitrogenase activity in legume root nodules, though total nitrogenase activity measurement is  
258 not possible (Vessey 1994). ARA was determined in pea roots collected when maximum  
259 nodules were observed, and in rice roots collected at maximum tillering stage. Pea plants were  
260 uprooted and excess adhered soils were removed carefully. Entire roots with intact nodules of  
261 each plant were put in a glass bottle (volume 630 ml) and mouth was made airtight with  
262 rubber septum and 10% of the bottle's air space replaced by acetylene gas (C<sub>2</sub>H<sub>2</sub>, >99.99%  
263 purity) and incubated at room temperature for 1 h. For determination of nitrogenase activity in  
264 rice roots, the entire roots of a rice hill was uprooted and separated from the above ground  
265 plant parts. The entire root was rinsed with standing water on same spot in the field to remove  
266 excess adhered mud and immediately placed in a glass bottle (volume 630 ml) and mouth was  
267 made airtight with rubber septum. An air volume of 10% of the bottle's air space was replaced  
268 by injecting acetylene gas (C<sub>2</sub>H<sub>2</sub>, >99.99% purity). Bottles were incubated at room  
269 temperature for 16 h at dark (Barraquio et al. 1986). Ethylene production was measured on a  
270 gas chromatogram (GC Top series 8000, CE instruments, Italy) by standard procedure and

271 nitrogenase activity expressed in  $\mu\text{mole of C}_2\text{H}_4 \text{ h}^{-1} 100 \text{ cc}^{-1}$  root volume (for rice) and  $\mu\text{mole}$   
272 of  $\text{C}_2\text{H}_4 \text{ h}^{-1} \text{ plant}^{-1}$  (for pea) (Thakuria et al. 2004).

273

274 Soil and plant sampling and analyses

275

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

284 Grain and straw/stover were sampled randomly on five plants from each plot at  
285 harvest for N, P and Zn uptake analysis. Plant samples were washed with 0.01 N HCl  
286 followed by several washings with de-ionized water and oven dried at 65 °C to constant  
287 weight. Samples were ground in a Willey Laboratory Mill. Tissue N was determined by  
288 micro-Kjeldahl digestion, distillation and titration procedures (Bremner and Mulvaney 1982).  
289 Ground tissue was digested in a mixture of  $\text{HNO}_3:\text{HClO}_4$  (3:1) and concentrations of P and Zn  
290 were determined by the ammonium molybdate (Olsen and Sommers 1982) and atomic  
291 absorption spectrophotometer (Perkin Elmer Analyst 200, USA), respectively.

292 Soil samples were analysed for pH (1:2 soil/water suspension) using a standard pH  
293 meter (Mettler Toledo, Model SevenEasy pH, GmbH, Switzerland), total N (by Kjeldahl  
294 method: digestion with concentrated  $\text{H}_2\text{SO}_4$  in presence of  $\text{K}_2\text{SO}_4$  and Zn dust at 360 °C in a  
295 Kjel Plus block digester, distillation in presence of excess NaOH and ammonia absorption in

296 boric acid; these steps were done by using Kjel Plus, Pelican Equipments, India. Then residual  
297 boric acid was titrated with standard 0.01 N H<sub>2</sub>SO<sub>4</sub> and total P by digestion in HNO<sub>3</sub>:HClO<sub>4</sub>,  
298 3:1, as described (Olsen and Sommers 1982). Available P (Bray's P) in soil was determined  
299 by stannous chloride blue color method (Bray and Kurtz 1945). Available Zn in soil was  
300 extracted by using DTPA as described by Liang and Karamanos (1993) followed by  
301 determination using atomic absorption spectrophotometer (Perkin Elmer Analyst 200, USA).  
302 Total organic C content in soil was determined by the dichromate oxidation method (Nelson  
303 and Sommers 1982). Soil aggregate analysis was done by wet sieving method (Camberdella  
304 and Elliott 1992). A 100 g soil sample (capillary-rewetted) was wet sieved by Yodder's  
305 apparatus through a series of sieves to obtain four size fractions: >2000 µm, 2000-250 µm,  
306 250-53 µm and <53 µm. Aggregate fractions retained on each sieve transferred to glass  
307 beaker and oven dried at 65 °C for weight determination.

308

### 309 Soil biological properties

310

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

1  
2  
3  
4 321 BBC, a mixture of bacterial inhibitors Rifampicin, Ampicillin, Chloramphenicol, Gentamycin  
5  
6 322 and Streptomycin, each had a final concentration of 1 mg g<sup>-1</sup> soil. These inhibitors were added  
7  
8 323 to samples by mixing with talc powder (Bailey et al. 2002). Each soil sample was sub-divided  
9  
10 324 into three equal sub-samples (20 g each). The control sub-sample received only talc @ 20 mg  
11  
12 325 g<sup>-1</sup> soil. Other two soil sub-samples received fungal and bacterial inhibitors, separately. The  
13  
14 326 treatment mixtures were thoroughly mixed and incubated at 25<sup>0</sup>C for 1 h. Then 1.0 ml of  
15  
16 327 0.4% glucose solution was added to each treatment tube, mixed thoroughly and inserted a  
17  
18 328 glass test tube containing 5 ml of 0.5 N NaOH in each sample vial and stoppered with rubber  
19  
20 329 bungs and re-incubated. Amount of CO<sub>2</sub> absorbed by NaOH was determined by titrating  
21  
22 330 against standard 0.1 N H<sub>2</sub>SO<sub>4</sub>. Rest calculations were done as per the procedure followed for  
23  
24 331 MBC determination. The MBC, FBC and BBC were expressed in terms of µg g<sup>-1</sup> dry soil.  
25  
26  
27  
28

29 332 Earthworms' casts were counted in plots at the start of the 3<sup>rd</sup> year crop cycle by  
30  
31 333 quadrature method. Plots were puddled, leveled and waited for the thin layer of water to  
32  
33 334 disappear 7 days before transplanting of 7<sup>th</sup> crop, *Sali* rice, 2004. On the leveled plots, the  
34  
35 335 earthworm casts appeared overnight and these casts were counted using 1 m<sup>2</sup> grid.  
36  
37  
38  
39

40 337 DNA extraction, polymerase chain reaction (PCR) and DGGE  
41  
42  
43  
44

45 339 Microbial DNA was extracted in freshly collected soil samples (500 mg) using the  
46  
47 340 commercial FastDNA Spin Kit for soil (BIO101, Vista, CA). The soil DNA content ranged  
48  
49 341 from 49.4 to 137.1 µg g<sup>-1</sup> dry soil. Partial 16S rRNA gene fragments in the sample DNA were  
50  
51 342 amplified using bacterial primers set described by Muyzer et al. (1993). Each amplification  
52  
53 343 reaction (50 µl) contained 50 ng soil DNA, 1U *Taq* DNA polymerase, 200 µM dNTPs, in a 10  
54  
55 344 mM TrisHCl buffer (pH 8.0), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl and 0.1% Triton X-100 and 32.5 p  
56  
57 345 mol of each primer. Template DNA was omitted from negative control reaction. All  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 346 amplifications were performed at least twice for each DNA sample obtained from each  
5  
6 347 replicate INM plot using a Hybaid Omnigene Thermocycler (Omnigene, The Netherlands)  
7  
8 348 and reaction conditions were maintained as described by Muyzer et al. (1993). Amplified  
9  
10 349 products were initially checked in agarose gel (1.5 % w v<sup>-1</sup>).

11  
12  
13 350           Amplified products were loaded on 8% acrylamide gel using a denaturant gradient of  
14  
15 351 45-65% and run at 75 V, 60 °C constant temperature for 18 h (Ingeny Phor mutation detection  
16  
17 352 system (Ingeny International BV, The Netherlands). Gel was stained with ethidium bromide  
18  
19 353 (0.5 mg L<sup>-1</sup>) and visualized under UV light on an Imago imaging system (Imago Scientific  
20  
21 354 Instruments, USA). Number of bands in each profile was recorded. The relative intensity of a  
22  
23 355 specific band was expressed as the ratio between the intensity of that band and the total  
24  
25 356 intensity of DNA in a profile. Pair-wise similarity matrix among DGGE profiles based on  
26  
27 357 numbers and relative abundances of DGGE bands using Dice correlation coefficient (Dice  
28  
29 358 1945) was determined. Hierarchical cluster analysis was performed using unweighted pair  
30  
31 359 group method with arithmetic means (UPGMA) on similarity matrix to construct dendrogram  
32  
33 360 to illustrate the relationship between bacterial community profiles of different nutrient  
34  
35 361 management plots.

36  
37  
38  
39  
40 362

41  
42 363 Statistical analysis

43  
44 364

45  
46  
47 365 All statistical analyses were performed using SPSS v. 12.0 (SPSS Inc. Chicago, IL). We  
48  
49 366 checked normality distribution among data generated from all replicated plots under six  
50  
51 367 nutrient management treatments for each parameter using the Kolmogorov-Smirnov test and  
52  
53 368 found normally distributed. For every parameter reported in this investigation, the six nutrient  
54  
55 369 management treatments were analysed for differences among means ( $P < 0.05$ ) by performing

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

372

373

## 374 **Results and Discussion**

375

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

377

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

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

1  
2  
3  
4 395 low organic C status ( $<8.0 \text{ g kg}^{-1}$  soil) and very low NUE of applied inorganic fertilizers in  
5  
6 396 these soils (Talukdar et al. 2004). During 3 years crop cycles, average increase in grain yield  
7  
8 397 of *Sali* rice over control plots was 21.5%, 18.6% and 29.7%, of *Ahu* rice 33.8%, 33.3% and  
9  
10 398 46.2% and in pod yield of the legume 254.6%, 266.2% and 296.7% due to application of  
11  
12 399 Urea:SSP:MOP, compost and bioinoculants based INM treatments, respectively (Table 2).  
13  
14  
15 400 This clearly indicated that response of legume crop (December-March) to applied nutrients  
16  
17 401 was highest followed by *Ahu* (April-July) and *Sali* rice (August-November) in RLR rotation.

18  
19  
20 402 Harvest index (HI) of grain crops refers to the ratio of grain yield by total biomass  
21  
22 403 yield (Rosielle and Frey 1975) and hence, HI can serve as a quality index for N management  
23  
24 404 in cropping system. Harvest index of rice crops in Urea:SSP:MOP and urea added PSB alone  
25  
26 405 INM plots was significantly ( $P<0.05$ ) lower than HI in Azo/Rh plus PSB dual INM and  
27  
28 406 Azo/Rh alone INM plots (Fig. 1). The higher quantity of straw production was responsible for  
29  
30 407 the lower HI of rice crops in Urea:SSP:MOP and urea added PSB alone INM plots, which  
31  
32 408 might be due to more availability of inorganic N through urea at early stages of crop growth.  
33  
34 409 Aulakh et al. (2000) also reported that an excess supply of inorganic N at the early stages of  
35  
36 410 crop growth encourages more vegetative growth. Overall, these results indicated that combine  
37  
38 411 application of compost, Azo/Rh and PSB along with PR and MOP sustain higher yields under  
39  
40 412 RLR rotation in acidic rice soils.

41  
42  
43  
44  
45 413

46  
47 414 Nitrogenase activity as influenced by bacterial bioinoculants based INM

48  
49  
50 415

51  
52 416 Nitrogenase activity in roots of rice and pea (intact nodules) was determined in all nine crops  
53  
54 417 except *Sali* rice, 2001 and French bean, 2001-02 (Fig. 2). In Azo/Rh alone INM and Azo/Rh  
55  
56 418 plus PSB dual INM plots, nitrogenase activity was significantly ( $P<0.01$ ) higher compared to  
57  
58 419 that of other treatments. The ability to fix atmospheric N by the test Azo strain in rice roots

1  
2  
3  
4 420 and also the synergistic effect of co-inoculation of Azo with PSB strain on N<sub>2</sub> fixation either  
5  
6 421 *in-vitro* or in field conditions were previously reported (Thakuria 2006). Similar to  
7  
8 422 observation by earlier workers (Roper and Ladha 1995), we also found that application of  
9  
10 423 compost stimulated nitrogenase activity in both rice and pea roots. In contrast, inorganic  
11  
12 424 fertilizer and urea included INM retarded nitrogenase activity but not significantly. Whether  
13  
14  
15 425 the stimulation or retardation in nitrogenase activity is associated with a corresponding  
16  
17 426 stimulation/retardation of population of the N<sub>2</sub> fixing microorganisms can not be confirmed as  
18  
19 427 we did not determine their population in soil. A very interesting observation was gradual  
20  
21 428 increase in nitrogenase activities in roots of rice and pea under Azo/Rh alone INM or Azo/Rh  
22  
23 429 plus PSB dual INM plots towards later crop cycles, which could be a result of either  
24  
25 430 population build up of introduced bioinoculants in the rhizosphere or better soil environment  
26  
27 431 for the N<sub>2</sub> fixers (Fig. 2). Although we observed persistence of these test strains in rice soil up  
28  
29 432 to one year after inoculation (Boro et al. 2004), their population was not monitored yearly in  
30  
31 433 this study. However, earlier research indicated that the counts of inoculated strains of  
32  
33 434 *Azospirillum* and *Azotobacter* increased 2 to 3 folds in pearl millet rhizosphere when  
34  
35 435 inoculation was continued for 3 years in fields with a corresponding increase in grain yield,  
36  
37 436 nitrogenase activity and N assimilation (Wani et al. 1988).  
38  
39  
40  
41  
42  
43  
44

#### 438 Nitrogen uptake and balance

45  
46  
47 439  
48  
49 440 Nitrogen removed by grains of six crops plus straw of *Ahu* rice 2003 in different nutrient  
50  
51 441 management plots differed significantly ( $P<0.05$ ; Table 3) after completion of the 2<sup>nd</sup> year  
52  
53 442 crop cycle. Nitrogen removed by six crops in control plots was the least (92 kg ha<sup>-1</sup>). In  
54  
55 443 compost and Urea:SSP:MOP plots, removal of N increased by 43.7% and 51% over control  
56  
57 444 plots, respectively and in bacterial bioinoculants based INM plots removal of N further  
58  
59  
60  
61  
62  
63  
64  
65



1  
2  
3  
4 445 increased by 7.2-14.7% and 12.7-20.5% over Urea:SSP:MOP and compost treated plots,  
5  
6 446 respectively. Higher grain yields and N concentration in grain/pod and straw/stover of RLR  
7  
8 447 rotation under bacterial bioinoculants based INM plots indicate more availability of N for  
9  
10 448 crop uptake (Table 2 and 3). These results support a positive role for N<sub>2</sub> fixation by the test  
11  
12  
13 449 Azo and Rh strains in rice and pea crops, respectively.

14  
15 450 Among three different bacterial bioinoculants based INM treatments, there was no  
16  
17 451 statistically significant difference observed in the amount of N removed, despite of ~2 fold  
18  
19 452 more N inputs added to soil in the urea added PSB alone INM treatment (Table 3).  
20  
21 453 Surprisingly, in this treatment, the apparent N loss was approximately double the amount of  
22  
23 454 apparent N loss observed in 0-15 cm soil depth in Azo/Rh plus PSB based INM or Azo/Rh  
24  
25 455 alone INM plots (Table 3). But this higher apparent N loss could not be accounted for  
26  
27 456 corresponding N removal value and apparent N gain in 15-30 cm soil depth suggesting N loss  
28  
29 457 in relatively higher amount from soil of this treatment. This resulted in a low agronomic  
30  
31 458 efficiency of applied N i.e. 8.5 kg grain kg<sup>-1</sup> N in urea added PSB alone INM plots as against  
32  
33 459 14.1 kg grain kg<sup>-1</sup> N in other two bioinoculants based INM plots. These results also suggest a  
34  
35 460 positive role for nitrogen fixation in soils under Azo/Rh based INM treatments. The  
36  
37 461 nitrogenase activity was also higher in these plots (Fig. 2).

38  
39 462 The data on apparent N loss in 0-15 cm and apparent N gain in 15-30 cm soil depth  
40  
41 463 under different treatments are in conformity with values of previous 14 reports that included  
42  
43 464 211 N balance values in rice based cropping system, out of which ninety-five percent values  
44  
45 465 were in between -60 to +90 kg N ha<sup>-1</sup>crop<sup>-1</sup> (Roger and Ladha 1992). The apparent loss of N  
46  
47 466 at 0-15 cm depth not only supports differential N uptake by the six crops in different nutrient  
48  
49 467 management treatments but also the movement of N from surface layer (0-15 cm) to the sub-  
50  
51 468 surface layer (15-30 cm) as evident from positive soil total N balances at 15-30 cm depth.  
52  
53 469 This data clearly indicated that application of N inputs in excess either as inorganic or organic  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 470 or in combination enhances loss of N from the system. Soils of the Brahmaputra basin are  
5  
6 471 highly weathered and light in texture, and intense rain in the region seems to cause leaching of  
7  
8 472 substantial amount of N from the surface layers particularly when the urea is a component of  
9  
10 473 external nutrient inputs. Excessive use of N fertilizers is known to promote nitrate leaching  
11  
12 474 (Aulakh et al. 2000; Ju et al. 2007). Under this situation, application of a bacterial  
13  
14 475 bioinoculant based INM appeared to enhance N assimilation by crops, nitrogen fixation in soil  
15  
16 476 and ability of the system to reduce N loss. The mechanisms of such beneficial effects of  
17  
18 477 bacterial bioinoculants based INM approach need to be addressed in future research.  
19  
20  
21

22 478

23  
24 479 Phosphorus uptake and balance

25  
26 480

27  
28 481 Phosphorus removed by grains of six crops plus straw of *Ahu* rice 2003 in control plots was  
29  
30 482 the least i.e. 22.8 kg ha<sup>-1</sup> (Table 3). In compost alone and Urea:SSP:MOP plots, removal of P  
31  
32 483 increased by 51.3% and 68.4% (significant at  $P<0.05$ ) over control plots, respectively.  
33  
34 484 Removal of P by six crops in bacterial bioinoculants based INM plots was significantly higher  
35  
36 485 ( $P<0.05$ ) compared to that in control or compost alone plots. Quantity of P removed in  
37  
38 486 bacterial bioinoculants based INM plots was 7.6-12% higher over that in Urea:SSP:MOP  
39  
40 487 plots (Table 3). These results indicated better P assimilation by the crops in bacterial  
41  
42 488 bioinoculants based INM plots under RLR rotation in acidic soils.  
43  
44  
45

46  
47 489 After harvest of six crops, a net negative soil total P balance at 0-15 cm depth ranged  
48  
49 490 from -26.4 to -61.3 kg P ha<sup>-1</sup> (Table 3). The depletion of total P from the initial value at 0-15  
50  
51 491 cm soil depth can not be justified by the amount of total P removed by the six crops and  
52  
53 492 suggests that two years of cultivation caused downward movement of P in soil as evident  
54  
55 493 from a net positive soil total P balance of +28.1 to +88.1 kg P ha<sup>-1</sup> at 15-30 cm soil depth  
56  
57 494 irrespective of nutrient management (Table 3). Zhang et al. (2003) earlier also reported that  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 495 substantial quantity of molybdenum reactive P can move down from surface layers to deeper  
5  
6 496 depth in paddy soils. We observed two distinct phenomena in relation to P management either  
7  
8 497 through PSB with PR or SSP in acidic soil. The Azo/Rh plus PSB dual INM and PSB alone  
9  
10 498 INM plots (both nutrient treatments received PSB with PR as source of P) showed least  
11  
12 499 apparent loss of soil total P i.e. 1.6 and 0.8 kg ha<sup>-1</sup>, respectively; whereas the Urea:SSP:MOP  
13  
14 500 and Azo/Rh alone INM plots (both nutrient treatments received SSP as source of P) showed  
15  
16 501 apparent gain in soil total P i.e. 11.4 and 6.0 kg ha<sup>-1</sup>, respectively at 15-30 cm depth after  
17  
18 502 harvest of six crops (Table 3). Therefore, application of readily soluble SSP in whole quantity  
19  
20 503 as basal dose in light textured acidic soil under high rainfall areas might encourage  
21  
22 504 translocation of substantial quantity of P to subsurface layer (15-30 cm soil depth). This  
23  
24 505 leached down soluble form of P immediately bound by highly active Fe and Al oxides at sub-  
25  
26 506 surface soil layer and thereby contributed a positive apparent soil total P balance. On the other  
27  
28 507 hand, PR is insoluble in soil and with time slowly dissolves through various microbial  
29  
30 508 mediated mechanisms (Kucey et al. 1989) and such available P fraction in soil was readily  
31  
32 509 taken up by plants and thereby less chance of leaching losses to deeper depth and hence no  
33  
34 510 positive apparent soil total P balance at 15-30 cm soil depth. The higher content of Bray's P in  
35  
36 511 soil (significant at  $P<0.05$ ) with corresponding higher quantities of P removal by crops under  
37  
38 512 bacterial bioinoculants based INM plots also supported the positive role of the test PSB strain  
39  
40 513 for better P assimilation by crops in RLR rotation (Table 3 and 5). The ability to solubilise tri-  
41  
42 514 calcium phosphate *in-vitro* and enhancement of rice growth and yield under field condition by  
43  
44 515 the test PSB strain was previously reported (Thakuria et al. 2004).  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

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

518

1  
2  
3  
4 519 Changes in soil DTPA extractable Zn in different nutrient management treatments after  
5  
6 520 completion of 1<sup>st</sup> year crop cycle and also uptake and balance of Zn for the *Sali* rice 2002 are  
7  
8 521 presented in the Table 4. The three bacterial bioinoculants based INM plots showed a  
9  
10 522 significant ( $P<0.05$ ) increase in soil DTPA extractable Zn content compared to that in control,  
11  
12 523 Urea:SSP:MOP and compost plots (Table 4). A high correlation coefficient ( $r=0.83$ ,  $P<0.01$ )  
13  
14 524 between grain yields and DTPA extractable Zn contents in soil after 1-year RLR rotation  
15  
16 525 indicated better Zn use efficiency in bacterial bioinoculants based INM plots compared to  
17  
18 526 other treatment plots. Zinc removed by grain and straw of *Sali* rice 2002 was significantly  
19  
20 527 ( $P<0.05$ ) higher in bacterial bioinoculants based INM plots compared to the amount of Zn  
21  
22 528 removed in control or compost plots. In control and compost alone plots, high apparent gain  
23  
24 529 of DTPA extractable Zn in soil after harvest of *Sali* rice 2002 and lower quantity of Zn  
25  
26 530 removed by that crop indicate the possibility of applied  $ZnSO_4$  getting transformed to  
27  
28 531 unavailable forms of Zn (clay-lattice bound, organic complexed, amorphous and crystalline  
29  
30 532 sesquioxides-bound, Hazra et al. 1987) in soil of these plots. Such transformation of applied  
31  
32 533  $ZnSO_4$  may also occur in INM plot soils but there is the possibility of solubilization of the  
33  
34 534 bound fractions of Zn in the bioinoculants based INM plots. Hence, uptake of Zn by plant in  
35  
36 535 these bioinoculants based INM plots was significantly ( $P<0.05$ ) higher (Table 4). However,  
37  
38 536 this need to be systematically investigated in future research. Raj (2002) also reported Zn-  
39  
40 537 solubilization by a *Bacillus* sp in soil and improvement in grain yield and Zn uptake of rice.  
41  
42 538 Therefore, improved Zn assimilation by the crops in bacterial bioinoculants based INM plots  
43  
44 539 argues against a specific effect of the bioinoculants on N or P nutrition in cropping system.  
45  
46  
47  
48  
49  
50  
51  
52  
53

540

54 541 Changes in total organic C content, aggregation, bacterial and fungal biomass, casting  
55  
56 542 activities of earthworms and in composition of bacterial communities of soil  
57  
58

543

1  
2  
3  
4 544 After completion of 2 years crop cycles, soil total organic C content depleted (ranged from 1.1  
5  
6 545 to 14.8%) at 0-15 cm depth and gained (ranged from 5.6 to 25.0%) at 15-30 cm soil depth  
7  
8 546 across all nutrient management plots compared to initial total organic C content values in the  
9  
10 547 respective soil depths (Table 5). Prior to this experiment, the field was lying fallow for 5  
11  
12 548 years. Therefore, this decline in total organic C at top layer might be associated with the  
13  
14 549 cultivation induced factors (Dalal and Mayer 1986). Earlier, Naklang et al. (1999) observed  
15  
16 550 depletion of soil organic C and labile C in 0-10 cm depth and gain of labile C in 20-40 cm  
17  
18 551 depth following conversion of forest land to rainfed rice in light texture soil. However, the  
19  
20 552 decline in soil total organic C was less in bacterial bioinoculants based INM plots and the  
21  
22 553 reason could be better soil aggregation. Bacterial bioinoculants based INM plots showed  
23  
24 554 significant ( $P<0.05$ ) higher water-stable aggregation and mass of 2000-250  $\mu\text{m}$  and 250-53  
25  
26 555  $\mu\text{m}$  soil aggregates at 0-15 cm soil depth compared to that in control, Urea:SSP:MOP and  
27  
28 556 compost alone plots after harvest of six crops in RLR rotation (Table 5). Mass of soil in  
29  
30 557 aggregates class  $<53 \mu\text{m}$  increased significantly ( $P<0.05$ ) in control, Urea:SSP:MOP and  
31  
32 558 compost plots compared to that in bacterial bioinoculants based INM plots. The formation and  
33  
34 559 stabilization of macro-aggregates (250-2000  $\mu\text{m}$ ) and micro-aggregates (53-250  $\mu\text{m}$ ) in these  
35  
36 560 plots perhaps physically protected higher amount of particulate organic matter and hence, less  
37  
38 561 chance of depletion of labile organic C from the surface layer (0-15 cm depth) to deeper soil  
39  
40 562 depths. These macro- and micro-aggregates contain higher amounts of particulate and light  
41  
42 563 fraction organic matters and that support higher rate of C and N mineralization in soils  
43  
44 564 (Manna et al. 2005; Yan et al. 2007). Our results also indicate higher rate of C and N  
45  
46 565 mineralization in bioinoculants based INM plots. However, laboratory incubation studies  
47  
48 566 using soils both from the bioinoculants based INM and other treatment plots need to be  
49  
50 567 carried out to generate data on C and N mineralization in future. Nevertheless, it is clear from  
51  
52 568 the data that straw/stover incorporation with inorganic fertilizers or compost in light texture  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 569 alluvial soils might not be sufficient to counter balance the loss of soil organic C and  
5  
6 570 deterioration of soil aggregation due to intensive cultivation under RLR rotation.  
7

8 571 The Azo/Rh plus PSB dual INM and Azo/Rh alone INM and compost plots supported  
9  
10 572 significant ( $P<0.05$ ) higher BBC compared to that in control, Urea:SSP:MOP and urea added  
11  
12 573 PSB alone INM plots (Fig. 3). In contrast, the control, Urea:SSP:MOP and urea added PSB  
13  
14  
15 574 alone INM plots supported significant ( $P<0.05$ ) high FBC than the other nutrient management  
16  
17 575 plots (Fig. 3). Population of bacteria and fungi determined in soils maintained a high  
18  
19 576 correlation co-efficient ( $r = 0.87$ ,  $P<0.01$ ) with BBC and FBC, respectively in corresponding  
20  
21 577 nutrient management plots (data not shown). The reflection of high MBC in control,  
22  
23 578 Urea:SSP:MOP and urea added PSB alone INM plots was due to exceptional high  
24  
25 579 contribution by FBC; FBC/BBC ratios were 3, 4.5 and 2.0, respectively in those plots. The  
26  
27 580 high FBC/BBC ratios in Urea:SSP:MOP and urea added PSB alone INM plots might be due  
28  
29 581 to lowering of soil pH, which stimulated fungal population significantly in those plots. After  
30  
31 582 harvest of six crops, the maximum pH drop (0.24 units) observed in Urea:SSP:MOP plots  
32  
33 583 followed by 0.20 units drop in urea added PSB alone INM plots (Table 5). The least pH drop  
34  
35 584 (0.05 units) was observed in Azo/Rh plus PSB dual INM plots. Bååth and Anderson (2003)  
36  
37 585 reported that fungal/bacterial ratio decreased significantly with increasing pH from about 9 at  
38  
39 586 pH 3 to approximate 2 at pH 7.0. Although, reduction in pH in soils of control plots was not  
40  
41 587 significant (Table 5), high FBC/BBC ratio in control plots could be a result of poor quality  
42  
43 588 rice straw incorporation in soil (low nutrient content) and lower quantity of legume stover  
44  
45 589 returned to the plots per RLR rotation. It has been reported earlier that low quality substrates  
46  
47 590 (high C/N) favor fungi while high quality (low C/N) substrates favor bacteria in soil (Bossuyt  
48  
49 591 et al. 2001). Thus we see that the bacterial bioinoculants based INM practice in RLR rotation  
50  
51 592 ensure annual high return of quality legume stover along with rice straw and compost and  
52  
53 593 associated biological N<sub>2</sub> fixation, consequently an improved status, uptake and balance of N  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 594 in soil (Table 3). As expected these factors caused an approximate balance of FBC/BBC ratio  
5  
6 595 ranging from 1.1 to 1.3 in bacterial bioinoculants based INM plots that in turn helped to  
7  
8 596 sustain a better nutrient mineralization process fueled by the labile C substrates in those plots.  
9  
10 597 Higher N, P and Zn assimilation by crops coupled with high content of soil available N, P and  
11  
12 598 Zn pools also supports the onset of a better mineralization process under bacterial  
13  
14 599 bioinoculants based INM practice. Thus the fractionation of the MBC to FBC and BBC, and  
15  
16 600 interpretation and use of their ratio in this study is justified as an index of better C and N  
17  
18 601 mineralization process in soil. Because, MBC is a measure of biomass that is size not activity  
19  
20 602 of microorganisms and therefore, use of MBC as a rapid indicator of C and N mineralization  
21  
22 603 processes in soil may be misleading. Earlier, Witt et al. (1998) also reported that MBC  
23  
24 604 measurement was a poor indicator of N mineralization–immobilization dynamics in soils.  
25  
26 605 Overall, results of FBC/BBC ratios suggested that incorporation of legume and rice crop  
27  
28 606 residues into soils along with external application of compost and bacterial bioinoculants  
29  
30 607 under rice based rotation were useful in maintaining balance between C and N dynamics in  
31  
32 608 soils. A balance between C and N dynamics in soils ensures more labile pools of soil organic  
33  
34 609 C, and hence better mineralization processes of nutrients in soils.  
35  
36  
37  
38  
39

40 610 After completion of 2 years crop cycles, earthworms' castings approximately doubled  
41  
42 611 (significant at  $P<0.05$ ) in Azo/Rh plus PSB dual INM and compost plots compared to that in  
43  
44 612 Urea:SSP:MOP, SSP added Azo/Rh alone INM and urea added PSB alone INM plots (Table  
45  
46 613 5). Rice field earthworms are endogeics and preferentially feed on high quality soil organic  
47  
48 614 matters. Endogeic earthworms preferably assimilate C from recently deposited fractions of  
49  
50 615 soil organic matter, which is composed of more readily decomposable substances (Edwards  
51  
52 616 and Arancon 2004). The bacterial bioinoculants based INM plots contained higher levels of  
53  
54 617 readily decomposable substances like particulate organic matter due to higher percentage of  
55  
56 618 macro- and micro-aggregates (Table 5). Again continuity of foods (for example more labile C  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4 619 and N pools in the soil) through out the year attracted more earthworms in compost and  
5  
6 620 bacterial biofertilizers based INM plots. However, addition of either urea or SSP reduced  
7  
8 621 casting activities of earthworms in Azo/Rh alone INM, PSB alone INM and Urea:SSP:MOP  
9  
10 622 plots. Though we do not know the exact cause for this reduction in casting activities of rice  
11  
12 623 field earthworms, previous study reported that earthworm numbers and biomass were  
13  
14 624 significantly less in inorganic fertilizer applied plots compared to manure-amended plots  
15  
16  
17 625 (Whalen et al. 1998), which needs further confirmation.

18  
19  
20 626 Hierarchical cluster analysis based on pair-wise similarity matrix of DGGE profiles  
21  
22 627 showed that bacterial community (in terms of number of bands and their relative abundances)  
23  
24 628 in control plots distinctly separated from the bacterial communities in other nutrient  
25  
26 629 management plots (Fig. 4). The four DGGE bands (indicated by horizontal lines with square  
27  
28 630 pointers in the lane 1, Fig. 4A) might represent succession of unique bacterial groups those  
29  
30 631 are specifically abundant in nutrient poor soils. The variation in DGGE banding pattern in  
31  
32 632 control plots compared to other nutrient management plots might be associated with  
33  
34 633 preferential colonization of rice straw in soil by certain groups of bacteria under poor N  
35  
36 634 availability condition, or otherwise decrease or elimination of abundant bacteria (for example  
37  
38 635 DGGE bands indicated by the horizontal lines with arrow pointers in the lane 1, Fig. 4A)  
39  
40 636 those prefer to colonize high quality soils, which needs further confirmation. Among nutrient  
41  
42 637 input added plots, 2 distinct clusters at  $\geq 82\%$  similarity level were formed by the bacterial  
43  
44 638 communities; one cluster represented by bacterial communities ( $>90\%$  similarity) in  
45  
46 639 Urea:SSP:MOP and urea added PSB alone INM plots and other one represented by the  
47  
48 640 bacterial communities ( $>99\%$  similarity) in Azo/Rh plus PSB dual INM and Azo/Rh alone  
49  
50 641 INM plots (Fig. 4B). Except control plots, the differences of bacterial communities among  
51  
52 642 nutrient input added plots were mainly due to change in relative band intensities, which  
53  
54 643 indicates shift in abundances of dominant bacterial groups. Interestingly, C and N dynamics in  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1  
2  
3  
4 644 soils between Azo/Rh plus PSB dual INM and Azo/Rh alone INM plots were comparable, and  
5  
6 645 soils of these two plots also harboured highly similar bacterial communities. Like-wise, C and  
7  
8 646 N dynamics, bacterial community compositions in soils between Urea:SSP:MOP and urea  
9  
10 647 added PSB alone INM plots were comparable. These findings implied that nutrient inputs  
11  
12 648 such as legume and rice crops residues, bacterial bioinoculants, inorganic fertilizers added in  
13  
14 649 different combinations to nutrient management plots modified community composition of soil  
15  
16 650 bacteria through their direct influence on C and N dynamics.  
17  
18  
19  
20  
21  
22  
23

24 651

25 652

26 653 **Conclusions**

27 654

28 655 Our study clearly demonstrated the multiple benefits of combined use of Azo/Rh with either  
29  
30 656 PSB plus PR or SSP, MOP and compost in sustaining higher yields, better N, P and Zn  
31  
32 657 assimilation by crops and improved soil quality under RLR rotation in acidic soil. These  
33  
34 658 bacterial bioinoculants based INM formulations with incorporation of crop residues of RLR  
35  
36 659 rotation in rainfed cropping system promoted more biological nitrogen fixation, better soil  
37  
38 660 aggregation and earthworm activities and thereby regulated a better C and N dynamics in  
39  
40 661 soils. The fungal/bacterial biomass C ratio in soil was found to be a better index of C and N  
41  
42 662 dynamics for measurement of short-term changes in acidic soil under RLR rotation. Results of  
43  
44 663 changes in bacterial community compositions in our experiment revealed the need of future  
45  
46 664 study to investigate impacts of continuous use of bioinoculants on microbial community  
47  
48 665 structure and its relation with soil functioning in cropping systems. We conclude that the INM  
49  
50 666 formulation containing compost, *Azospirillum/Rhizobium* with either phosphate-solubilizing  
51  
52 667 bacteria plus phosphate rock or SSP and MOP emerges as a superior nutrient management  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

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

670

671

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

675

676

## 677 **References**

678

679 Aulakh MS, Khera TS, Doran JW, Singh K, Singh B (2000) Yields and nitrogen dynamics in  
680 a rice-wheat system using green manure and inorganic fertilizer. *Soil Sci Soc Am J*  
681 64:1867-1876

682 Bååth E, Anderson TH (2003) Comparison of soil fungal/bacterial ratios in a pH gradient  
683 using physiological and PLFA-based techniques. *Soil Biol Biochem* 35:955-963

684 Bailey VL, Smith JL, Bolton H (2002) Fungal to bacterial ratios in soils investigated for  
685 enhanced C sequestration. *Soil Biol Biochem* 34:997-1007

686 Barraquio WL, Daro MLG., Tirol AC, Ladha JK, Watanabe I (1986) Laboratory acetylene  
687 reduction assay for field grown wetland rice plants. *Plant Soil* 90:359-372

688 Boro RC, Goswami C, Thakuria D, Modi MK, Talukdar NC (2004) Molecular and functional  
689 characteristics, growth promoting effect and persistence of selected parent isolates and  
690 streptomycin resistant derivatives of rice rhizobacteria. *Ind J Exp Biol* 42:1186-1194

691 Bossuyt H, Deneff K, Six J, Frey SD, Merckx R, Paustian K (2001) Influence of microbial  
692 populations and residue quality on aggregate stability. *Appl Soil Ecol* 16:195-208

- 1  
2  
3  
4 693 Bray RH, Kurtz LT (1945) Determination of total, organic and available forms of phosphorus  
5  
6 694 in soils. *Soil Sci* 59:39-45  
7  
8 695 Bremner JM, Mulvaney CS (1982) Nitrogen-total. In: Page AL, Miller RH, Keeney DR (eds)  
9  
10 696 *Methods of Soil Analysis, Part 2- Chemical and Microbiological properties*. 2<sup>nd</sup>  
11  
12 697 Edition, Agron Monogr 9, ASA, SSSA, CSSA, Madison, WI, pp 595-623  
13  
14  
15 698 Cáceres EAR (1982) Improved medium for isolation of *Azospirillum* species. *Appl Environ*  
16  
17 699 *Microbiol* 44:990-998  
18  
19  
20 700 Camberdella CA, Elliott ET (1992) Particulate organic matter changes across a grassland  
21  
22 701 cultivation sequence. *Soil Sci Soc Am J* 56:777-783  
23  
24 702 Choudhury ATMA, Kennedy IR (2004) Prospects and potentials for systems of biological  
25  
26 703 nitrogen fixation in sustainable rice production. *Biol Fertil Soils* 39:219-227  
27  
28  
29 704 Dalal RC, Mayer DG (1986). Long-term trends in fertility of soils under continuous  
30  
31 705 cultivation and cereal cropping in southern Queensland. II. Total organic carbon and  
32  
33 706 its rate of loss from the soil profile. *Aust J Soil Res* 24:281-292  
34  
35  
36 707 DeDutta SK (1989) Integrated nutrient management in relation to soil fertility in lowland rice-  
37  
38 708 based cropping systems. In: *Rice Farming Systems: New Directions*. Proceedings of  
39  
40 709 an International Symposium 31 January–3 February 1987. International Rice Research  
41  
42 710 Institute, Manila, Philippines, pp. 141-160  
43  
44  
45 711 Dice LR (1945) Measures of the amount of ecological association between species. *Ecology*  
46  
47 712 26:297-302  
48  
49  
50 713 Dobermann A, White PF (1999) Strategies for nutrient management in irrigated and rainfed  
51  
52 714 lowland rice systems. *Nutr Cycl Agroecosys* 53:1-18  
53  
54 715 Edwards CA, Arancon NQ (2004) Interactions among organic matter, earthworms, and  
55  
56 716 microorganisms in promoting plant growth. In: Magdoff F, Weil RR (eds) *Soil*  
57  
58 717 *Organic Matter in Sustainable Agriculture*, CRC Press, UK, pp. 328-363  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 718 Fernández LA, Zalba P, Gómez MA, Sagardoy MA (2007) Phosphate-solubilization activity  
5  
6 719 of bacterial strains in soil and their effect on soyabean growth under greenhouse  
7  
8 720 conditions. *Biol Fertil Soils* 43:805-809  
9
- 10 721 George T, Ladha JK, Garrity DP, Buresh RJ (1994) Legume as 'nitrate catch' crops during  
11  
12 722 the dry to wet transition in lowland rice cropping systems. *Agron J* 86:267-273  
13  
14 723 Hafeel K, Rate AW, Abbott L (2004) Calibration of the substrate induced respiration and  
15  
16 724 selective inhibition techniques for fungal bacterial ratios in Western Australian soils.  
17  
18 725 SuperSoil 2004:3<sup>rd</sup> Australian New Zealand Soils Conference, 5-9 December,  
19  
20 726 University of Sydney, Australia, <http://www.regional.org.au/au/asssi/>, pp 1-6  
21  
22  
23  
24 727 Hazra GC, Mandal B, Mandal LN (1987) Distribution of Zinc fractions and their  
25  
26 728 transformation in submerged rice soils. *Plant Soil* 104:175-181  
27  
28  
29 729 Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil.  
30  
31 730 V. A method for measuring soil biomass. *Soil Biol Biochem* 8:209-213  
32  
33  
34 731 Jeyabal A, Kuppaswamy G (2001) Recycling of organic wastes for the production of  
35  
36 732 vermicompost and its response in rice-legume cropping system and soil fertility. *Euro*  
37  
38 733 *J Agron* 15:153-170  
39
- 40 734 Johri BN, Sharma A, Viridi JS (2003) Rhizobacterial diversity in India and its influence on soil  
41  
42 735 and plant health. *Adv Biochem Engin/Biotechnol* 84:49-89  
43  
44  
45 736 Ju XT, Kou CL, Christie P, Dou ZX, Zhang FS (2007) Changes in soil environment from  
46  
47 737 excessive application of fertilizers and manures to two contrasting intensive cropping  
48  
49 738 systems on the North China Plain. *Environ Pollut* 145:497-506  
50  
51  
52 739 Khan A, Chandra D, Nanda P, Singh S, Ghorai A (2004) Integrated nutrient management for  
53  
54 740 sustainable rice production. *Arch Agron Soil Sci.* 50:161-165  
55  
56  
57 741 Khan SA, Mulvaney RL, Ellsworth TR, Boast CW (2007) The myth of nitrogen fertilization  
58  
59 742 for soil carbon sequestration. *J Environ Qual* 36:1821-1832  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 743 Kucey RMN, Janzen HH, Leggett ME (1989) Microbially mediated increase in plant-  
5  
6 744 available phosphorus. *Adv Agron* 42:199-228  
7  
8 745 Ladha JK, Reddy PM (2003) Nitrogen fixation in rice systems: state of knowledge and future  
9  
10 746 prospects. *Plant Soil* 252:151-167  
11  
12 747 Liang J, Karamanos RE (1993) DTPA-extractable Fe, Mn, Cu and Zn. In: Carter MR (ed) *Soil*  
13  
14 748 *Sampling and Methods of Analysis*, Canadian Society of Soil Science, Lewis  
15  
16 749 Publishers, Canada, pp. 87-90  
17  
18 750 Lucy M, Reed E, Glick B R (2004) Applications of free living plant growth-promoting  
19  
20 751 rhizobacteria. *Antonie van Leeuwenhoek* 86:1-25  
21  
22 752 Manna MC, Swarup A, Wanjari RH, Singh YV, Ghosh PK, Singh KN, Tripathi AK, Saha  
23  
24 753 MN (2005) Soil organic matter in a West Bengal inceptisol after 30 years of multiple  
25  
26 754 cropping and fertilization. *Soil Sci Soc Am J* 70:121-129  
27  
28 755 Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations  
29  
30 756 by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-  
31  
32 757 amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695-700  
33  
34 758 Naklang K, Whitbread A, Lefroy R, Blair G, Wonprasaid S, Konboon Y, Suriya-arunroj D  
35  
36 759 (1999) The management of rice straw, fertilizers and leaf litter in rice cropping  
37  
38 760 systems in Northeast Thailand. 1. Soil carbon dynamics. *Plant Soil* 209:21-28  
39  
40 761 Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In: Page  
41  
42 762 AL, Miller RH, Keeney DR (eds.) *Methods of Soil Analysis. Part 2- Chemical and*  
43  
44 763 *Microbiological properties*, 2<sup>nd</sup> Edition, *Agron Monogr* 9: 961-1010, ASA, SSSA,  
45  
46 764 CSSA, Madison, WI , pp. 539-594  
47  
48 765 Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeney DR (eds)  
49  
50 766 *Methods of Soil Analysis, Part 2- Chemical and Microbiological properties*. 2<sup>nd</sup>  
51  
52 767 Edition, *Agron Monogr* 9, ASA, SSSA, CSSA, Madison, WI, pp 403-430  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 768 Palaniappan SP (1985) Cropping system in the tropics. Wiley Eastern Ltd., New Delhi, India,  
5  
6 769 pp 215  
7  
8 770 Pampolino MF, Manguiat IJ, Ramanathan S, Gines HC, Tan PS, Chi TTN, Rajendran R,  
9  
10 771 Buresh RJ (2007) Environmental impact and economic benefits of site-specific  
11  
12 772 nutrient management (SSNM) in irrigated rice systems. *Agr Syst* 93:1-24  
13  
14  
15 773 Prasad R, Nagarajan S (2004) Rice–wheat cropping system – Food security and sustainability.  
16  
17 774 *Curr Sci* 87:1134-1135  
18  
19  
20 775 Raj A (2002) Biofertilizers for micronutrients. *Biofertilizers Newsletter* 10:8-10  
21  
22 776 Reddy PR, Raju AP (2006) Integrated nutrient management for rice. Proceedings of 18<sup>th</sup>  
23  
24 777 World Congress of Soil Science, 166-20: 4.2A Soil Care and Quality Soil  
25  
26 778 Management, July 9-15, Philadelphia, Pennsylvania, USA  
27  
28  
29 779 Regmi AP, Ladha JK, Pathak H, Pasuquin E, Bueno C, Dawe D, Hobbs PR, Joshy D, Maskey  
30  
31 780 SL, Pandey SP (2002) Yield and soil fertility trends in a 20-year rice-rice-wheat  
32  
33 781 experiment in Nepal. *Soil Sci Soc Am J* 66:857-867  
34  
35  
36 782 Roger PA, Ladha JK (1992) Biological N<sub>2</sub>-fixation in wetland rice fields: Estimation and  
37  
38 783 contribution to nitrogen balance. *Plant Soil* 141:41-55  
39  
40  
41 784 Roper MM, Ladha JK (1995) Biological N<sub>2</sub> fixation by heterotrophic and phototrophic  
42  
43 785 bacteria in association with straw. *Plant Soil* 174:211-224  
44  
45 786 Rosielle AA, Frey KJ (1975) Estimates of selection parameters associated with harvest index  
46  
47 787 in oat lines derived from a bulk population. *Euphytica* 24:121-131  
48  
49  
50 788 Somado EA, Becker M, Kuehne RF, Sahrawat KL, Vlek PLG (2003) Combined effects of  
51  
52 789 legumes with rock phosphorus on rice in West Africa. *Agron J* 95:1172-1178  
53  
54  
55 790 Srivastava SC, Singh JS (1989) Effect of cultivation on microbial carbon and nitrogen in dry  
56  
57 791 tropical forest soil. *Biol Fertil Soils* 8:343-348  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 792 SubbaRao NS (1999) Soil Microbiology. 4th edition of Soil Microorganisms and Plant  
5  
6 793 growth, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi  
7  
8 794 Sundara Rao WVB, Sinha MK (1963) Phosphate dissolving organisms in the soil and  
9  
10 795 rhizosphere. Ind J Agric Sci 33:272-278  
11  
12 796 Talukdar NC, Bhattacharyya D, Hazarika S (2004) Soils and agriculture of Brahmaputra  
13  
14 797 Basin. In: Singh VP, Sharma N (eds) Brahmaputra Basin Water Resources. Kluwer  
15  
16 798 Academic Publishers, Holland, pp 94-112  
17  
18 799 Talukdar NC, Chakravarty DN (1988) Effects of varying levels of N, P and K on grain yield  
19  
20 800 and nutrient uptake of *Ahu* rice in a light texture soil under high annual rainfall. Ann  
21  
22 801 Agric Res 9:159-164  
23  
24 802 Tewari SN, Chakraborty DN, Bora PK (1969) Retention and transformation of soluble  
25  
26 803 phosphorus added to Assam (India) soils. J Inst Chemists 61:174-176  
27  
28 804 Thakuria D (2006) Interactive effect of direct and indirect plant growth promoting bacteria on  
29  
30 805 microbial community structure of rice rhizosphere. A Ph.D. Thesis. Department of  
31  
32 806 Biotechnology, Gauhati University, Assam, India  
33  
34 807 Thakuria D, Talukdar NC, Goswami C, Hazarika S, Boro RC, Khan MR (2004)  
35  
36 808 Characterization and screening of bacteria from rhizosphere of rice grown in acidic  
37  
38 809 soils of Assam. Curr Sci 86:978-985  
39  
40 810 Vessey JK (1994) Measurement of nitrogenase activity in legume root nodules: In defence of  
41  
42 811 the acetylene reduction assay. Plant Soil 158:151-162  
43  
44 812 Wani SP, Chandrapalaih S, Zambre MA, Lee KK (1988) Association between N<sub>2</sub> fixing  
45  
46 813 bacteria and pearl millet plants: Responses, mechanisms and persistence. Plant Soil  
47  
48 814 110:289-302  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4 815 Whalen JK, Parmelee RW, Edwards CA (1998) Population dynamics of earthworm  
5  
6 816 communities in corn agroecosystems receiving organic or inorganic fertilizer  
7  
8 817 amendments. *Biol Fertil Soils* 27:400-407  
9  
10 818 Witt C, Cassmann KG, Ottow JCG, Biker U (1998) Soil microbial biomass and nitrogen  
11  
12 819 supply in an irrigated lowland rice soils as affected by crop rotation and residue  
13  
14 820 management. *Biol Fertil Soils* 28:71-80  
15  
16  
17 821 Witt C, Gaunt JL, Galicia CC, Ottow JCG, Neue HU (2000) A rapid chloroform fumigation  
18  
19 822 extraction method for measuring soil microbial biomass carbon and N in flooded rice  
20  
21 823 soils. *Biol Fertil Soils* 30:510-519  
22  
23  
24 824 Yadav JSP (2003) Managing soil health for sustained high productivity. *J Ind Soc Soil Sci*  
25  
26 825 51:448-465  
27  
28 826 Yadvinder-Singh, Bijay-Singh, Ladha JK, Khind CS, Gupta RK, Meelu OP, Pasuquin E  
29  
30 827 (2004) Long-term effects of organic inputs on yield and soil fertility in the rice-wheat  
31  
32 828 rotation. *Soil Sci Soc Am J* 68:845-853  
33  
34  
35 829 Yan D, Wang D, Yang L (2007) Long-term effect of chemical fertilizer, straw, and manure on  
36  
37 830 labile organic matter fractions in a paddy soil. *Biol Fertil Soils* 44:93-101  
38  
39  
40 831 Zhang HC, Cao ZH, Shen QR, Wong MH (2003) Effect of phosphate fertilizer application on  
41  
42 832 phosphorous (P) losses from paddy soils in Taihu Lake Region. I. Effect of phosphate  
43  
44 833 fertilizer rate on P losses from paddy soil. *Chemosphere* 50:695-701  
45  
46  
47 834

48  
49 835 **Figure legends**

50  
51  
52 836  
53  
54 837 **Fig. 1** Harvest index of rice crops influenced by different nutrient management treatments in  
55  
56 838 rice-legume-rice rotation. Values that differ significantly (one-way ANOVA,  $P < 0.05$ ) within  
57  
58 839 each cluster of dendrograms are followed by different letters. Azo is *Azospirillum*; Rh is



1  
2  
3  
4 840 *Rhizobium*; PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-  
5  
6 841 phosphate; MOP is muriate of potash.

7  
8 842 **Fig. 2** Nitrogenase activity in roots of *Sali* rice (A), pea (B) and *Ahu* rice (C) influenced by  
9  
10 843 different nutrient management treatments under rice-legume-rice rotation. Nitrogenase  
11  
12 844 activity in roots of *Sali* rice and French bean of 1<sup>st</sup> year crop cycle were not determined. Each  
13  
14 845 value on the line graph represents mean nitrogenase activity in roots of 12 plants from four  
15  
16 846 replicated plots. Values that differ significantly (one-way ANOVA,  $P < 0.01$ ) on each line  
17  
18 847 graph are followed by different letters. Azo is *Azospirillum*; Rh is *Rhizobium*; PSB is  
19  
20 848 phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP is  
21  
22 849 muriate of potash.

23  
24  
25  
26 850 **Fig. 3** Microbial biomass C (MBC), bacterial biomass C (BBC) and fungal biomass C (FBC)  
27  
28 851 influenced by different nutrient management treatments determined after harvest of six crops  
29  
30 852 in rice-legume-rice rotation. Values that differ significantly (one-way ANOVA,  $P < 0.05$ )  
31  
32 853 within each parameter are followed by different letters. Azo is *Azospirillum*; Rh is *Rhizobium*;  
33  
34 854 PSB is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate;  
35  
36 855 MOP is muriate of potash.

37  
38 856 **Fig. 4** Denaturing gradient gel electrophoresis (DGGE) profiles of 16S rRNA gene fragments  
39  
40 857 obtained by PCR amplification using bacterial primer sets (Muyzer et al. 1993) in soils of  
41  
42 858 different nutrient management treatments. (A) an image of ethidium bromide stained DGGE  
43  
44 859 gel and (B) hierarchical cluster plot based on similarity matrix of DGGE profiles. Joints of  
45  
46 860 the branches of the dendrogram indicate the percentage similarity based on unweighted pair  
47  
48 861 group method with arithmetic means (UPGMA). Azo is *Azospirillum*; Rh is *Rhizobium*; PSB  
49  
50 862 is phosphate solubilizing bacteria; PR is phosphate rock; SSP is single super-phosphate; MOP  
51  
52 863 is muriate of potash.

53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Figure1

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

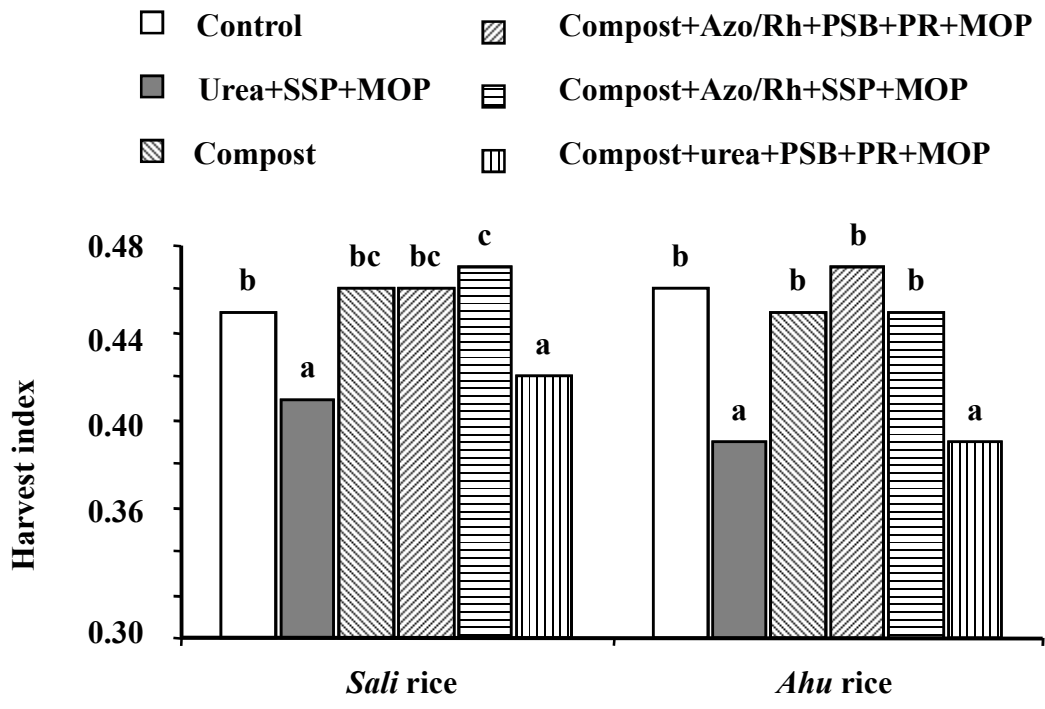
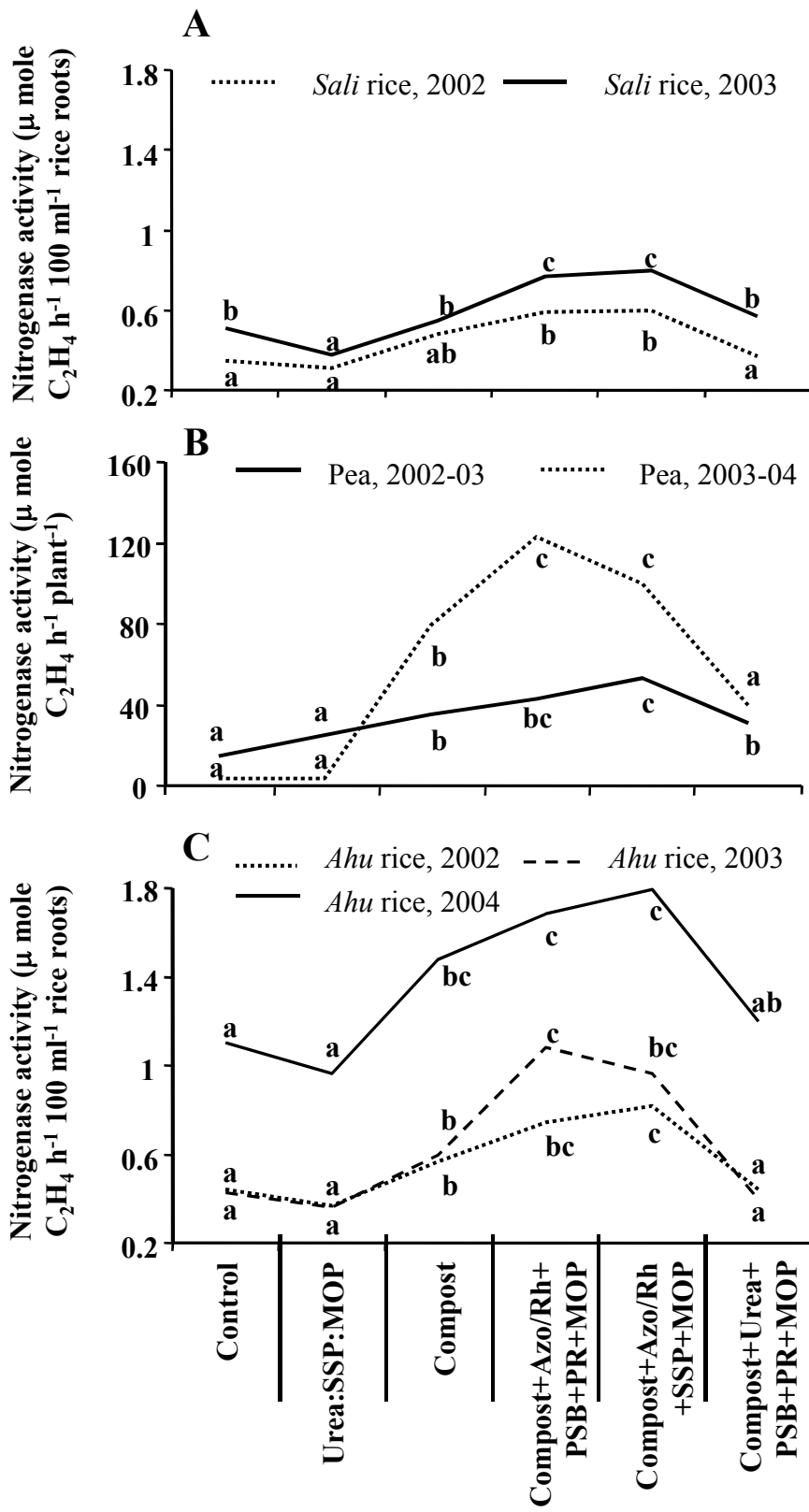


Figure2



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Figure3

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

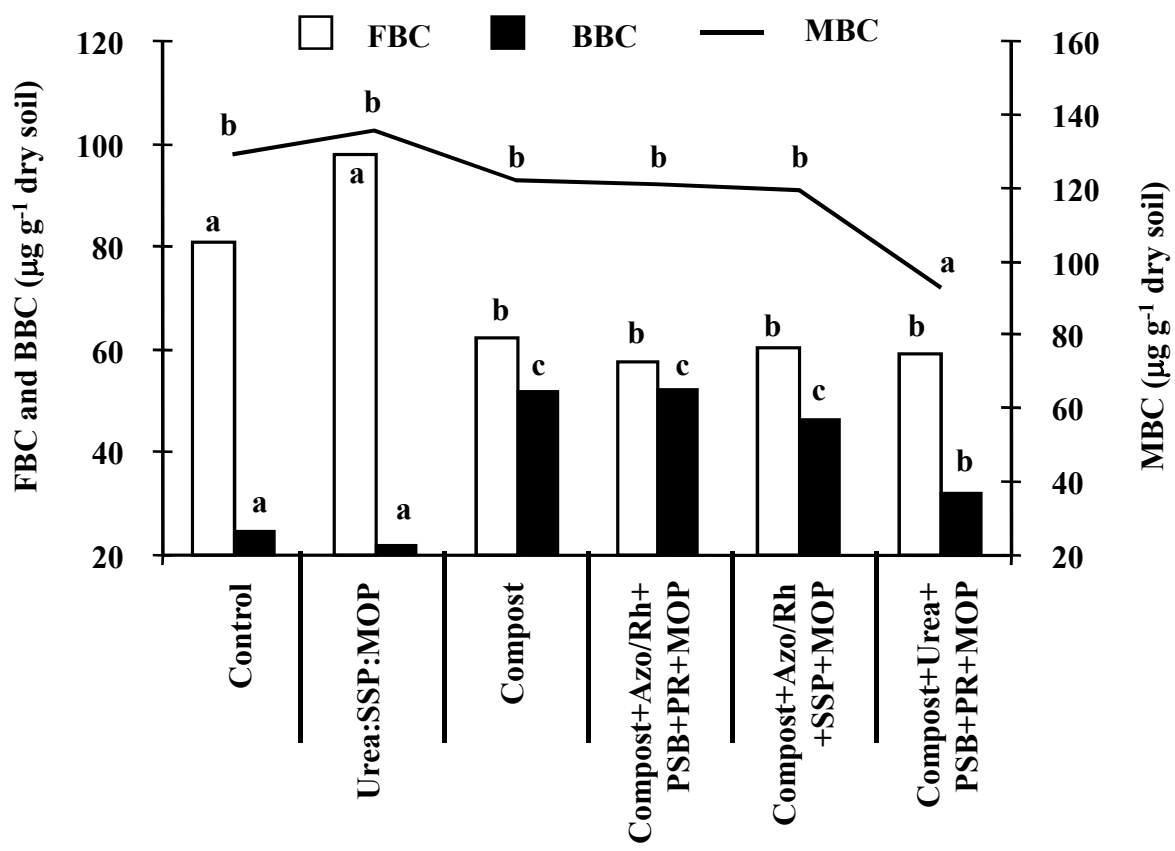
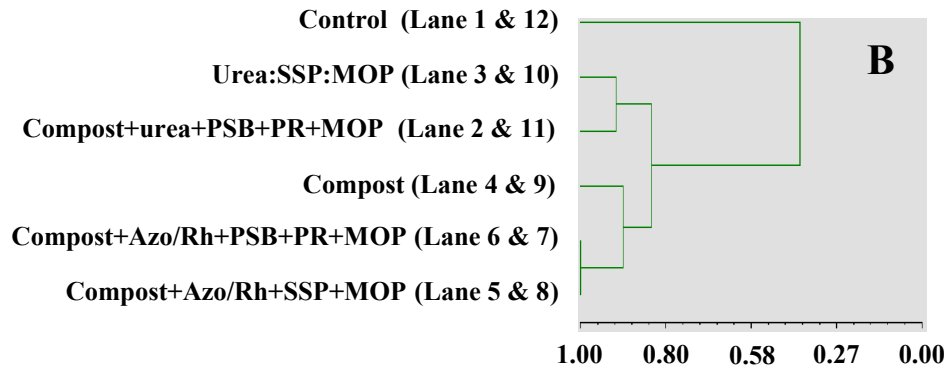
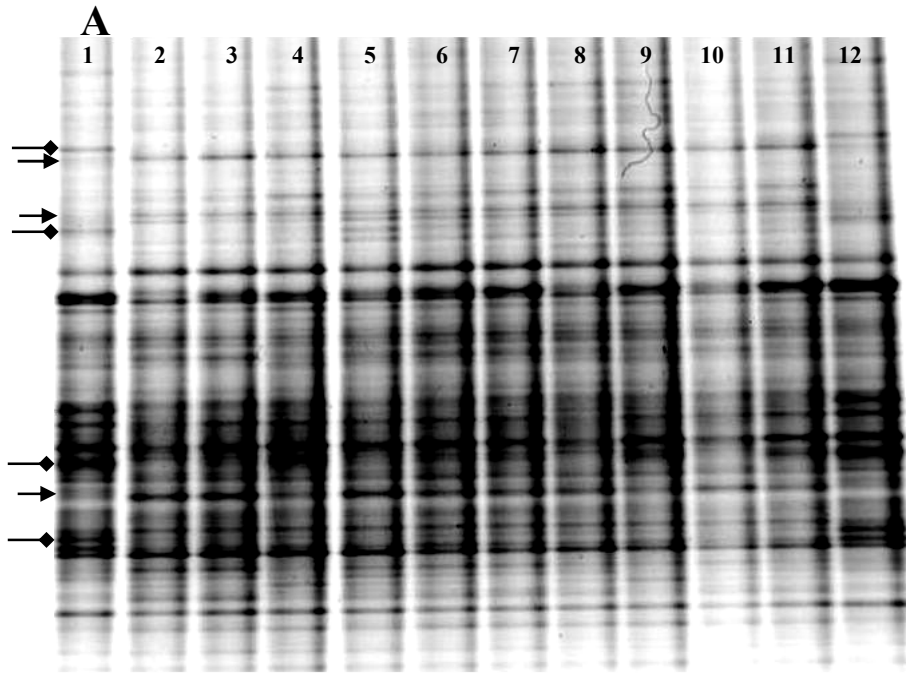


Figure4



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

**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 <sup>§</sup>	Form of fertilizer <sup>†</sup>	Compost (Mg ha <sup>-1</sup> )
1 <sup>st</sup> Year 2001-02	<i>Kharif</i>	<i>Sali</i> rice	Ranjit	Aug. 14	Dec. 09	40:20:20	Urea:SSP:MOP	3
	<i>Rabi</i>	French bean	Contender	Feb. 05	Apr. 05	20:30/15:20	Urea:SSP/PR:MOP	2
	<i>Summer</i>	<i>Ahu</i> rice	Luit	Apr. 20	July 11	40:20/10:20	Urea:SSP/PR:MOP	3
2 <sup>nd</sup> year 2002-03	<i>Kharif</i>	<i>Sali</i> rice	Ranjit	July 28	Nov. 28	40:20/10:20 and 5	Urea:SSP/PR:MOP and ZnSO <sub>4</sub>	3
	<i>Rabi</i>	Pea	Boneville	Nov. 15	Apr. 01	20:30/15:20	Urea:SSP/PR:MOP	2
	<i>Summer</i>	<i>Ahu</i> rice	Luit	Apr. 07	July 04	40:20/10:20	Urea:SSP/PR:MOP	3
3 <sup>rd</sup> year 2003-04	<i>Kharif</i>	<i>Sali</i> rice	Ranjit	Aug. 14	Dec. 10	40:20/10:20	Urea:SSP/PR:MOP	3
	<i>Rabi</i>	Pea	Azad	Nov. 19	Apr. 05	20:30/15:20	Urea:SSP/PR:MOP	2
	<i>Summer</i>	<i>Ahu</i> rice	Luit	Apr. 25	July 27	40:20/10:20	Urea:SSP/PR:MOP	3

<sup>§</sup>Applied rates were based on N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O:ZnSO<sub>4</sub> kg ha<sup>-1</sup>, ZnSO<sub>4</sub> was applied only to the *Sali* rice crop of the 2<sup>nd</sup> year crop cycle.

<sup>†</sup>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 1<sup>st</sup> 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<sup>§</sup>

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

<sup>§</sup>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<sup>-1</sup> 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.

**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<sup>§</sup>

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

<sup>§</sup>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.



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

<sup>§</sup> 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%;

<sup>†</sup> 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<sup>§</sup>

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

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

<sup>δ</sup> Values with - sign indicates unit drop from the initial soil pH 4.80.

<sup>†</sup> Values with - and + signs indicate loss and gain, respectively over the initial total organic C contents 8.8 and 6.7 g kg<sup>-1</sup> soil at 0-15 and 15-30 cm depth, respectively.

<sup>¶</sup> 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.