Research Results on Biological Nitrogen Fixation with the System of Rice Intensification

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In Madagascar, rice production still remains low with an average national yield of about 2 t/ha, despite governmental efforts to promote rice production over the past 50 years. These actions were based mainly on the use of chemical fertilizers and improved varieties, directed to the three or four major rice-producing areas in the country. Peasants represent 80% of the Malagasy population, and half of them own only about 0.25 to 0.30 hectare of rice field upon which they depend for their survival. Hence, increase in the productivity of land area is imperative for achieving food security and reducing poverty.

To bring solutions to small farmers who lack resources, and particularly financial resources, SRI is most promising. High yield attainment, up to 8 to 12, and even 16 tons per hectare, and plants' resistance to diseases observed during on-farm experiments are particular features of this system. It will be really significant if small farmers can produce over 500 kg paddy from 0.05 ha surface area with this system as this can contribute greatly to family food security.

With this system, farmers need only use farmyard manure and/or compost to get best results. This is a form of biological agriculture that benefits from the maximum action of soil microorganisms. We have seen that there is biological nitrogen fixation (BNF) associated with rice roots under SRI practices and have identified the role of *Azospirillium* as a source for meeting plant nitrogen needs. This report will present some findings from this research. It is hoped that the existence of BNF effects will reduce the quantity of N fertilizer required and utilized. With better understanding of these dynamics and effects we should be able to move toward a replacement of inorganic fertilization with biological means.

Acceptance of SRI by the scientific establishment in Madagascar has been promoted by several research projects undertaken since 1997 by the Faculty of Agriculture (Ecole Supérieure des Sciences Agronomiques, ESSA) at the University of Antananarivo. Research on rice cultivation needs to take into account the socio-economic context in the country. The government's policy to date intended to develop the country's "rice baskets," where there are large stretches of ricefields held by big farmers, has not brought any viable solution for Madagascar, which still imports huge quantities of rice to feed its population. Most peasants, 80% of the population, are still living below the threshold of poverty, growing rice just for survival.

Two major constraints need to be taken into account in rice research and development: the small size of most exploited areas per farmer, ranging up to 0.25-0.30 hectare, and the low purchasing power of farmers. Increasing productivity without dependence on the use of chemical fertilizers and other purchased inputs should be a goal at least for the short to middle term. Our research has focused on the support and the validation of SRI, motivated by the facts outlined above.

The main factors affecting yield in SRI include water management, mechanical weeding (using a hand rotating weeder), and transplantation of single, young seedlings. These practices have been experimented with on different kinds of soil (clay, loam or sand), repeated over several years in different parts of Madagascar. We hope to develop more precise knowledge on the effects of using the mechanical rotating weeder and other weeding methods such as use of herbicides that can be compared with one another.

We have been working recently on understanding the phenomena that can support biological nitrogen fixation to contribute to high yield. Thanks to the iso-

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lation and identification of *Azospirillium* sp., which is associated with high yields, we want to suggest a concept of "incitement" for enhanced BNF which manure and/or compost can achieve in conjunction with SRI methods with different soils and in different ecological regions.

Objectives of Experiments

The main purpose of our research is to demonstrate that the high performance of SRI is linked to the activities of aerobic microorganisms located in the rhizosphere and root system. Different yields have been evaluated according to soil texture, which can be clay, loam or sand, which can play a role in BNF capacity. Considering all of the yield increase factors in SRI, we find a lot of similarities with the fundamental principles of organic agriculture that were described by Boucher (1961).

Water management practices with SRI allow for better oxygenation of the root zone during the growth cycle, leading to better development of the aerobic microbial environment. Mechanical weeding using a hand "rotating hoe" with small toothed wheels, following transplantation of plants in a square or rectangular pattern, increases the number of soil pores so that roots and microorganisms can more easily gain access to oxygen. Research by Puard et al. (1986) has underscored the importance of oxygen for submerged lowland crops.

Experiments in soils managed according to SRI principles were expected to show the presence of *Azospirillium* in the root system of the rice plant, which is able to fix nitrogen from atmospheric sources, the quantity of which according to Ladha et al. (1998) can attain 55-70 kg/ha.

Methods and Materials

Multifactorial and multilocal experiments with SRI (N=288 and 240)

1. The main controlled variables related to the availability of water and nutrients:
   - 1a: **Water management** and the use of **compost,** contrasted with
   - 1b: **Water management** and the use of **NPK fertilizer**.

These were combined with agronomic practices affecting the rice plants themselves:
   - 2a: **Age of seedlings** at transplantation (8 days vs. 16 or 20 days),
   - 2b: **Number of seedlings** per hill (1 vs. 3), and
   - 2c: **Spacing** (25cm x 25cm vs. 30cm x 30cm).

The experiments were laid out in Fischer blocs repeated 3 times, with control plots that were:
   - 3: Without water management, i.e., with continuous submergence of the crop, and
   - 4: Without fertilization.

2. The first set of experiments was carried out near Morondava on the west coast of Madagascar, a second at Anjomakely, south of the capital city Antananarivo on the high plateau, and a third at Beforona near the east coast. In each location there were soils with different structures: clay, loam, balanced, and sand.

To assess the performance of SRI, as well as the efficiency of organic inputs, these trials were undertaken in different agroecological areas: with semi-arid climate at Morondava in the west; with temperate climate at Anjomakely on the high plateau in the center of the country; and with hot, humid weather around Beforona in the eastern part Madagascar toward the sea.

Experiments with different weeding methods (N=72)

The following variables were evaluated at the Anjomakely site:

1: **Weeding**:
   - 1a: **Mechanical weeding**, using a 'rotating hoe' (2, 3, or 4 times);
   - 1b: **Use of a herbicide** — 2,4-D;
   - 1c: **Hand weeding** (once) supplemented by use of the herbicide 2,4-D;
   - 1d: **Mulching** with *Sesbania* leaves (with layers 5cm thick); and
   - 1e: **Interplanting** of *Sesbania* in alley cropping.

In the trials, 1a, 1b, 1c, 1d and 1e were combined with other key SRI practices:

2: **Water management**; and

3: **Plant management** — transplantation of single seedlings 8 days old.

The trials were conducted with variations also in:

4: **Compost** — 3 doses of 2, 3 and 6 t/ha each, treating doses as the experimental variable rather than compost vs. NPK fertilizer vs. no fertilization.

The experiments are laid out in randomized blocs with three repetitions, with a control plot where hand weeding was done as needed. There was no systematic weeding; weeds were only gathered manually.

Experiments with various doses of compost and their effects on tillering (N=18)

The variables controlled here were:

1: **Use of compost** (1, 2, 3, 4, or 6t/ha); combined with

2: **Water management** as recommended for SRI;

3: **Plant management** — transplantation of single seedlings 8 days old**;** and

4: **Three weedings,** all compared with a control plot **without compost**.

Multilocal trials were carried out in the Antananarivo, Fianarantsoa and Moramanga regions.

Counting and identification of *Azospirillium* colonies

Azospirillium colonies were isolated from extracts of rhizosphere and root solutions at the end of the tillering period using a "milieu Döbereiner." A litre of the solution can contain KH_2PO_4 (0.4g), K_2HPO_4(0.1g), $MgSO_4$-$7H_2O$ (0.2g), NaCl (0.1g), $CaCl_2$ (0.02g), $FeCl_3$ (0,01g), $NaMoO_4$-$2H_2O$ (0.002g), fumarate (1.0g), glucose (1.0g), H_2O (q.s.p: 1l), and agar (15.5g).

The number of bacteria in the extract solution is assessed by a comparison with the table of Grady. Once reproduced, *Azospirillium* sp. can be tested in various crops (Krieg and Döbereiner, 1984; Magalhaes et al., 1983; Reinhold et al., 1987; Martinez-Drets et al., 1985).

	A. brasilense	*A. lipoferum*	*A. amazonense*	*A. balopraeferans*
Pleomorphic cells in alkaline media	–	+	–	+
Biotin requirement	–	+	–	+
Use of glucose	–	+	+	–
Use of sucrose, maltose, trehalose, lactose	–	–	+	–
Use of keto glutamate	–	+	–	+

Glucose = D-fructose, D-galactose, L-arabinose, lactate, malate, succinate, pyruvate and fumarate. We have used D-fructose and lactose to identify our colonies.

Results and Discussions

Importance of oxygenation and organic inputs with SRI

Oxygenation of soil resulting from controlled water management and from use of a rotating weeder contributes to the high performance of SRI. Zhang Xi et al. (1999) showed that layers of ferro-hydroxide appear on the surface of paddy-field water under anaerobic conditions. When roots have insufficient O_2, this limits their access to nutrients. This can be a serious problem in Madagascar where, given its pedology, the majority of soils are ferralitic. Plants can consequently be affected by iron toxicity and aluminium saturation, which can form with available P_2O_5 a complex that is largely irreversible.

Research done by Revsbech et al. (1999) has brought some important precision on the amount of O_2 found in the rice root system. Multifactorial experiments conducted at Morondava on the west coast where reddish sandy soils are irrigated, and in other regions on clay and loamy soil under rainfed conditions, have confirmed not only the high performance of SRI methods but also the positive effect on yields from the use of organic fertilizers.

In Table 1 on page 151 we classify yields obtained in these trials according to soil textures and the type of fertilizer used. The table shows the results from experiments conducted by Rajaonarison (1999) at Morondava on the west coast, Raobelison (2000) at Beforona in the east, and Andriankaja (2001) at Anjomakely, 18 km south of Antananarivo on the high plateau, testing our hypotheses.

Water management kept water depth lower than 10cm, with alternation of wetting-irrigation every 3 days during 40 days from transplantation to the end of the tillering period. Three weedings using rotary hand weeders were applied at regular intervals.

Under the same cultural conditions, the best yields, over 10 t/ha, were obtained in clay and balanced soils with the use of compost (organic fertilizer), while medium yields, 5.3-7.9 t/ha, were obtained without any fertilization. Loam and reddish sandy soils gave poorer yields.

Our trials on the variation of tiller number under the influence of different weeding methods consequently showed the possible benefits of oxygen infiltration into the soil that can invigorate the root system. Compared to other rice cultivation systems, root development as well as nutrient assimilation is better with SRI. (This is seen from the preceding paper.) This ad-

Table 1: Average yields according to soil type and fertilization

Variables	Yields according to type of soil (t/ha): Reddish Sand *(Morondava)*		Clay: Good soil *(Anjomakely)*		Loam: Poor soil *(Anjomakely)*		Balanced Soil *(Beforona)*	
Seedling age at transplanting (days)	8	20	8	16	8	16	8	20
No fertilizer	2.3	2.0	5.3	2.7	1.7	1.2	7.9	7.5
Fertilizers added:								
N P K	6.4	4.2	8.2	6.2	5.2	3.2	–	–
Compost	6.9	3.8	10.3	7.4	6.2	4.0	10.2	8.2

Table 2: Comparison of the structure of soils used for experiments

Ecological Areas	% Clay	% Loam	% Sand	Soil Structure
Morondava (semi-arid climate)	10-20	15-25	55-70	Sand
Anjomakely (temperate climate)	15-24	30-34	34-37	Loam
"	22-32	20-30	33-37	Clay
Beforona (humid warm climate)	30-34	34-38	34-40	Balanced

vantage is linked to the transplanting of very young seedlings, to water management that consists of alternately wetting and drying the rice paddies, and to early and frequent weedings with a hand weeder (rotary hoe).

This classification was done making a comparison between our soil analysis results and the soil texture table in *Le Memento de l'Agronome* (Ministère de la Cooperation Française, 1993).

Table 2 shows us that the three experimental areas each have different types of soil, which gives us some leads for understanding how the variation of soil oxygenation in each of them could affect plant performance. For loam, yields seem to be lower due to difficulties in oxygenation. This is linked to the fact that loam contains mostly small particles, which are more likely to settle after submersion and thus to limit soil porosity. Sands, although poor in nutrients, are actually moderately favorable to SRI according to our trials. The best oxygenation levels could be achieved when experiments were done on irrigated rice paddies that have very good water management, which made possible a doubling of rice production (Rajaonarison, 1999).

For clay with a larger proportion of sand, we could have the best yield since clay remains moist and retains water better, while sand contributes to drainage and soil aeration. An alternation of wetting and submersion as the pattern of water management leads to an increase in the exchange capacity of the soil, supported by the proportion of sand that allows infiltration after abundant rains.

Our observations on SRI are similar to ones by Wade et al. (1998) who explained the effects of water on the aeration of soils, as well as the loss of certain nutrients such as NO_3 (nitrate), according to differences in the soil structure and texture.

Oxygenation, as a result of SRI water management practices and repeated use of the rotating hoe with its wheels that aerate the top horizon of the soil, leads to better development of the rice root system. Armstrong and Webb (1985) made this same observation about the possibility of rice roots' extended growth under the influence of O_2. This root development becomes much more evident with SRI practices.

A measurement of root length by Raobelison (2000) showed lengths of 30-35 cm for SRI vs. 15-20 cm for traditional rice management. Research by Barison (2002) with 108 farmers who were using both SRI and traditional rice cultivation practices on their farms showed SRI rooting depth down to 50 cm, whereas traditionally grown rice had shallower rooting in soil that was kept saturated as much as possible rather than intermittently aerated, as with SRI.

Furthermore, Kirk and Solivas (1997) noted that "the upper limit of root length per soil volume is probably determined by transport of O_2 through the root and the ability of the root system to aerate itself." This is consistent with the results we see with SRI. Seedlings transplanted at 20 days have less root volume than those set out at 8 days. The latter have undergone less stress and have more favorable micro-organism development in, on and around the roots. The use of organic fertilizer like manure and compost that micro-organisms can easily degrade leads to the best yields when accompanied by other optimal cultural practices (Uphoff, 2001).

Tillering and yield variation according to weeding methods with SRI

Consideration of the effects of weeding using a hand rotating weeder, which oxygenates the rice paddy soil leads us to examine further the results of the factorial trials that used Fischer bloc design with 3 replications, so as to evaluate its effects on the evolution of tillering.

Our trials evaluating different weeding methods (Table 3) show that these methods can definitely affect tillering, and we think that the aeration of the soil makes a critical contribution for better development of the root system. This root development is very striking for SRI compared to the other systems of cultivation, giving plants easy access to nutrients. These results are due to the combined effects of transplanting very young plants (8 days), careful water management with alternate wetting and drying of the soil, and early and frequent weeding with a mechanical hand weeder.

There is a proportionality between the number of tillers and the number and frequency of weeding. The best yield was obtained with 4 weedings during the cycle of vegetative growth with rotary weeder. Yields can increase 1.2 t/ha from each use of the weeder. The use of *Sesbania* mulch is of less interest but still economic; it gives results similar to those with the use of the herbicide 2, 4D. This last can be compared with 2 times of weeding with the rotating weeder.

Note from Table 3, that when herbicide is used to control weeds, tillering is steady or even declines with an increase in the application of compost. This probably reflects an adverse effect of the herbicide upon the soil microbial populations that are otherwise stimulated by the addition of a greater amount of compost. Alternatively, the herbicide could have some direct effect upon the plant's growth performance.

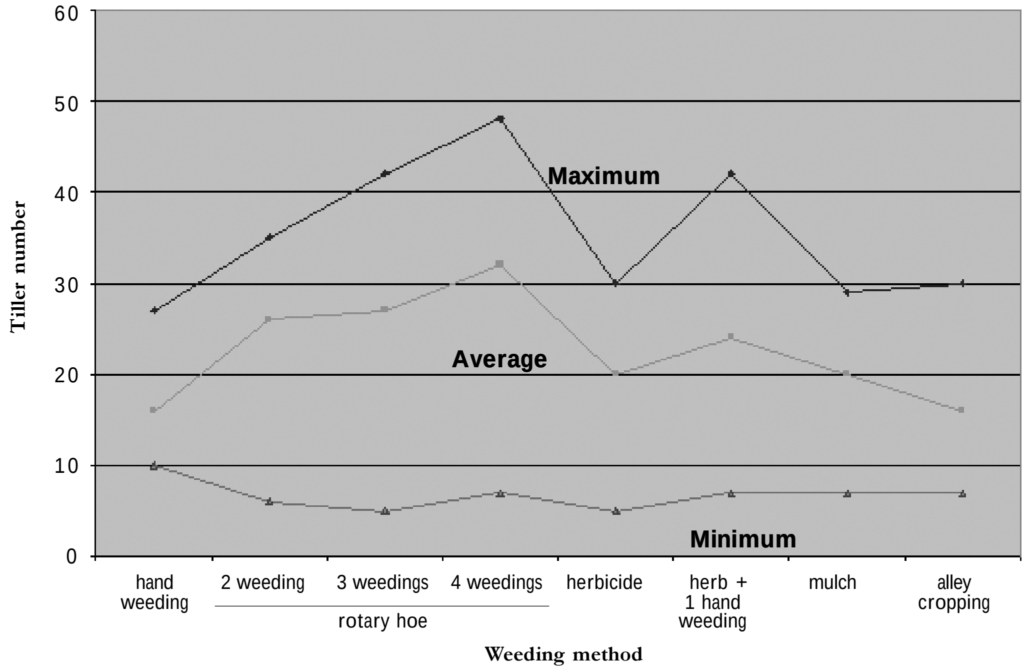
Yield data from these trials are analyzed in Table 4, showing tons per hectare for these weeding and compost practices varied with other SRI methods. The figures shown in tons per hectare are averaged for three plots that had different soil quality (two with better clay soils, and one with poorer loamy soil).

Table 3: Average minimum and maximum number of tillers by weeding methods and compost application with SRI

	Compost Doses								
	2 t/ha			3 t/ha			6 t/ha		
Variables	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min
Hand weeding as needed	27	16	10	29	15	12	29	16	12
Weeding with rotary hoe:									
2 times	35	26	6	40	29	12	42	24	5
3 times	42	27	5	50	30	11	46	32	12
4 times	48	32	7	58	31	10	58	34	8
Herbicides 2,4 D	30	20	5	31	19	7	31	18	9
Herbicides plus hand weeding	42	24	7	36	22	10	38	16	6
Sesbania mulch	29	20	7	30	17	6	36	16	5
Alley cropping with *Sesbania*	30	16	7	33	17	7	34	17	10

Conditions: single seedlings aged 8 days; water management alternating drying and wetting soil, 3 days each period, with 10cm water level at most. Research done in 2001-2002.

Figure 1. Effects of weeding method on tiller number



The benefits of soil aeration from using the rotating hoe are very evident from the data in Table 4. Compared to hand weeding on an as-needed basis as the baseline, 2 mechanical weedings added 1.5 t/ha (41%); 3 mechanical weedings added 2.4 t/ha (63%), and 4 mechanical weedings added 3.9 t/ha (104%), other things being equal. Use of weedicide added only 1.3 t/ha (34%), and one manual weeding with herbicide added only 1.6 t/ha (41%), about the same increase as from two mechanical weedings. Use of sesbania either as mulch or intercrop added little to yield compared to hand seeding, though using this leguminous species gave about 20% more yield on average with ust 2 t/ha of compost compared with adding 6 t/ha. A small amount of organic matter added to the soil interacted favorably with this other means for enriching the soil.

Determination of an 'incitement' threshold for BNF when using increasing doses of compost, evaluated from tillering

In Madagascar, the recommended doses of fertilizer for rice paddies are 10t/ha of manure/compost, or 150 kg/ha of 11N-22P-16K and 120 kg/ha of urea. Nevertheless, from experiments at Beforona by Raobelison (2000), we have noticed that the yields with SRI did not depend upon the amount of compost applied. This means that high doses of compost are not needed, suggesting instead that low fertilizer dose can achieve almost the same result (Table 5 and Figure 2).

Table 4: Yields (t/ha) with weeding methods and compost application with SRI

Weeding Methods	2 tons/ ha Compost	3 tons/ ha Compost	6 tons/ ha Compost	Ave.
Hand weeding	4.046	3.469	3.554	3.750
Rotary hoe:				
2 times	5.157	5.593	5.141	5.297
3 times	5.891	6.148	6.307	6.115
4 times	6.978	8.092	7.844	7.638
Herbicide	5.125	4.939	4.998	5.021
Herbicide plus one hand weeding	5.061	5.272	5.567	5.300
Mulching	4.421	3.142	3.800	3.790
Alley cropping	4.268	3.491	4.189	3.983
Average	5.119	5.041	5.175	5.112

Experiments were undertaken to determine the fertilizer dose that can be considered as sufficient for 'incitement' of *Azospirillium* to contribute to a level of

Figure 2. Relation between tiller numbers and yields with applications of straw

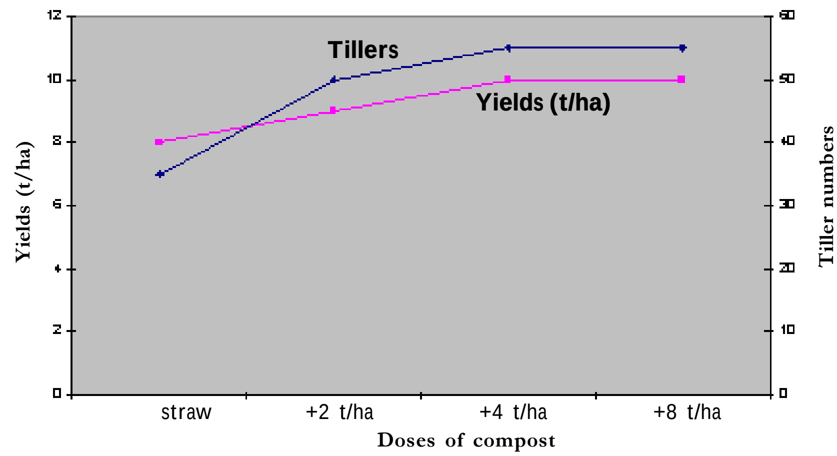


Table 5: Effects of increasing doses of compost on tillering

	8-day seedlings		20-day seedlings	
Treatments	Tillers (N)	Yields (t/ha)	Tillers (N)	Yields (t/ha)
Straw	20 - 35	7.9	20 - 35	7.5
Straw + manure 2t/ha.	45 - 50	9.0	40 - 45	8.0
Straw + manure 4t/ha.	50 - 55	9.8	40 - 45	8.1
Straw + manure 8t/ha.	50 - 55	10.2	40 - 45	8.2

Source: Raobelison (2000)

BNF, equivalent to 2 t/ha. This implies that a crop does not necessarily need a large amount of organic fertilizer to trigger a soil biological response.

A threshold for the amount of compost that can be considered as an 'incitement' for microorganism activity to accomplish BNF was determined from trials at Anjomakely, Beforona and Fianarantsoa (see Table 6 and Figure 3).

These results indicate an 'incitement' threshold for compost with SRI methods can be just 1 to 2 t/ha compared to the currently recommended doses of 5 to 10 t/ha, though younger seedlings gave better response to more compost applications than did older ones. This could be because with SRI plant, soil, water and management practices, the value of the compost is more indirect — for its triggering and sustaining soil microbial activity which produces or acquires nutrients for the plant, e.g., through P solubilization — than for its direct nutrient contributions assessed in terms of the amount of nutrients contained in the compost itself.

Assessing the effects of BNF with SRI

Stewart (1977) and Hamdi (1982) reported many years ago on the existence of different kinds of microorganisms in the soil (rhizosphere) that can fix nitrogen, residing around, on or in plant roots. These organisms can be either symbiotic or associative according to their fixation ability. If the nitrogen fixation occurs as a reduction of N_2 into NH_3, as in the case of rice plants, it is considered as associative fixation, and the energy for the reduction is provided either by carbohydrate from the soil or by root exudates. As a corollary, we can note that if carbohydrates are the dominant energy source, then fixation is low, and vice versa, fixation is higher if carbohydrates are not the dominant source of energy. This observation can be seen in Table 7.

Currently, our research is focusing on the importance of BNF to explain the high performance of SRI. We want to know whether the use of fertilizer in inorganic forms (NPK) as well as application of large amounts of such fertilizer will decrease, through competition from other microorganisms more benefited by the fertilizer, the number of *Azospirillium* that are located in the root system, which will then inhibit the process of BNF. The comparisons made are for:

Clay: no fertilizer, traditional cultivation, not SRI

Loam: no fertilizer, SRI

Clay: no fertilizer, SRI

Loam: with compost, SRI

Clay: with NPK, SRI

Clay: with compost, SRI

Table 6: Amounts of compost application indicating the 'incitement' threshold of microorganisms for fixing N and effects on tillering

	Average Number of Tillers								
	Anjomakely			Beforona			Fianarantsoa		
Compost applications	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min
0 t/ha	42	23	10	44	32	26	35	20	14
1 t/ha	46	25	13	50	37	31	39	25	18
2 t/ha	60	30	17	47	33	25	42	29	15
3 t/ha	55	31	15	-	-	-	40	34	20
4 t/ha	52	32	16	48	35	27	38	32	23
6 t/ha	49	35	12	50	38	29	-	-	-

Conditions: Single seedlings aged 8 days at transplanting; water management: alternate drying and wetting of 3 days each with 10cm water level at most. Research in 2001-2002.

Table 7. *Azospirillium* sp. counts according to treatments

Treatments		Number of *Azospirillium* sp.			
Texture/method	Fertilizer	Soil near root system (10^3/ml)	Roots (10^3/mg)	Yields (t/ha)	Tillers (N)
Clay/traditional	No	25	65	1.8	17
Loam/SRI	No	25	75	2.1	32
Clay/SRI	No	25	1,100	6.1	45
Loam/SRI	Compost	25	2,000	6.6	47
Clay/SRI	N P K	25	450	9.0	68
Clay/SRI	Compost	25	1,400	10.5	78

Notes: These trials were undertaken at Anjomakely in 2001 with alternate drying and wetting every 3 days from transplantation to the end of the tillering period, and with 3 weedings with a hand weeder.

Control comparisons: Traditional rice cultivation (non-SRI): clumps of 30-day-old seedlings, no water management, no weeder operation but weeding by hand.

Retirement of Azospirillium *was by using Döbereiner's method (cf. Reproduction and multiplication environment in method and materials), and the obtained number of* Azospirillium *was derived by comparisons with Grady's table.*

Plant sampling was done at the end of the tillering period.

The concentration of *Azospirillium* in the soil (rhizosphere) remains low and constant (n = 25x 10^3/ml) no matter what are the soil texture and the fertilizer level. Very significant differences can only be noticed in the root system. From n = 450 x 10^3/mg (for treatments with NPK), we can expect better yields and better tillering.

We find the number of *Azospirillium* sp. decreasing when chemical fertilizers are used (see Table 7 and Figure 4). This would explain the important need for nitrogen sources with other intensified systems of rice production such as the System of Rice Improvement (SRA), promoted by the Madagascar government. SRA demands two applications of urea (2 x 60 kg/ha), the first after transplanting, and the second before the end of tillering period.

Rice plants that are provided with chemical fertilizer have less colonies of *Azospirillium* sp (n = 450x10^3/

Figure 3. Compost Incitement, Anjomakely and Beforona

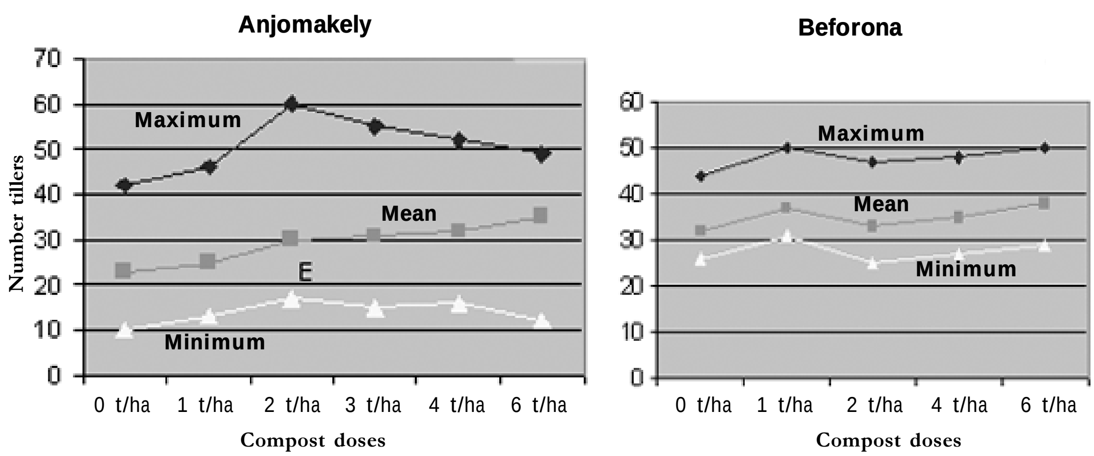
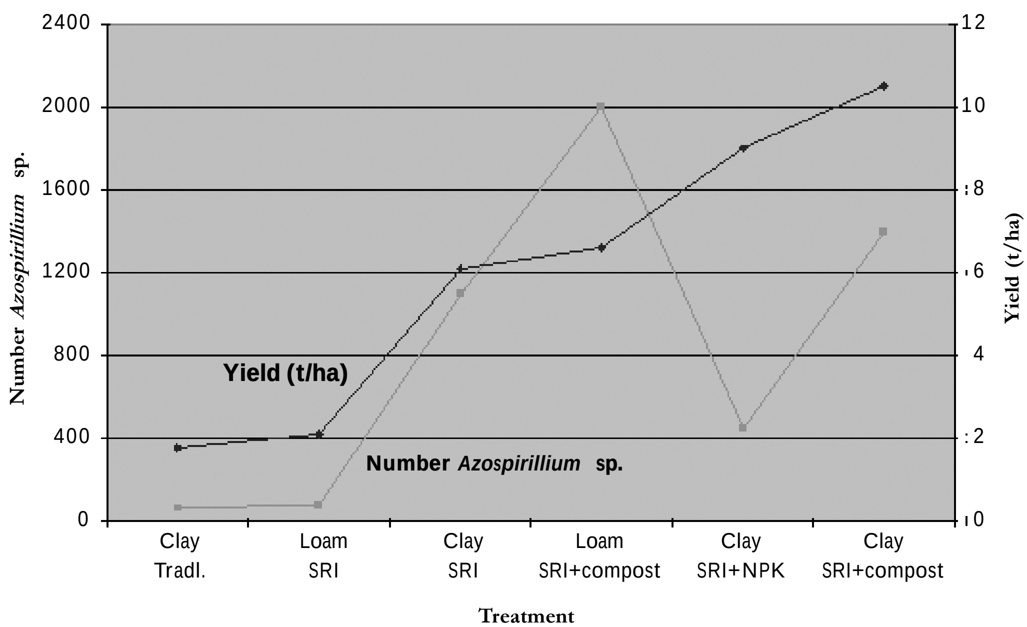


Figure 4. *Azospirillium* number according to treatments



mg), compared to those that were fertilized with compost (n = 1400x10^3/mg). Nevertheless, both of them brought the two highest yields (Figure 4). The three colonies that were retrieved from them and identified were: *A. brasilense*, *A. lipoferum*, and probably *A. amazonense*.

Discussion

Our trials consider SRI as a variant of organic agriculture practice. Use of compost and adapted seeds for each ecological region can give high yields with only a small dose of nutrients. Plants grown in on-farm SRI experiments were undamaged by diseases. Patterns of yield variation observed according to differences in the type of soil and the amount of fertilizer that was applied lead us to conclude that the reproduction and multiplication of microorganisms induced by inoculation of seeds or by compost amendments contributes to increased yield while cutting down on the need to use fertilizer. Further trials to establish a test for "effectiveness" regarding the consequences of soil and plant inoculation will be undertaken in future research.

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