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# Biodiversity and Sustainability of Wetland Rice Production: Role and Potential of Microorganisms and Invertebrates

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**ABSTRACT:** Some of the ecological foundations of sustainable wetland rice production related to microorganisms and invertebrates and their biodiversity are considered including: (i) aspects of sustainability of rice-producing environments involving microbial and invertebrate populations, the maintenance of soil fertility, effects and control of rice pests and vector-borne diseases; (ii) how crop intensification affects these populations and their biodiversity; (iii) agricultural practices that use microbial and invertebrate populations and their biodiversity; and (iv) the status of germplasm collections and the potential of biotechnology to use them to improve the sustainability of rice-producing environments.

The beneficial and detrimental roles of microorganisms and invertebrates in sustainable rice production have been identified and, sometimes, quantified. However, less is known about the possible long-term effects of crop intensification on these populations and their biodiversity. Numerous methods using microorganisms and invertebrates to increase soil fertility and control pests and diseases have been tested. But the success of these methods is limited and their adoption almost negligible. This will probably continue while the methods are still based on a very restricted knowledge of biodiversity, community structure, and trophic relationships at the ecosystem level.

Recent data on arthropods confirm that high biodiversity does not imply stability and low pest populations. Increases in pest and vector densities depend more on predator diversity, species resilience to perturbations, and biological attributes. Thus, increasing or preserving diversity *per se* does not necessarily contribute to pest stability, but developing effective trophic linkages might. This approach might also be valid for maintaining soil fertility through microbial management, the

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optimization of primary production in floodwater, and the optimization of nutrient recycling by invertebrate populations.

## **Introduction**

More than half of the world's population depends on rice which, in 1988, occupied 145 million hectares of land, with a global production of 468 million tonnes. An additional 300 million tonnes of rice will be needed in 2020 to meet the need of a fast-growing human population. This requires a 65% production increase within 30 years without much expansion of the actual cultivated area (International Rice Research Institute, 1989). However, increased rice production should not be at the expense of future generations and should fulfil the concept of sustainability. It should be achieved through rice production management that: (i) satisfies changing human needs and maintains production over time in the face of ecological difficulties and social and economic pressure; (ii) maintains or enhances the quality of the environment; and (iii) conserves or enhances natural resources. Aside from maintaining growth in productive agricultural systems and promoting growth in less productive systems, the major issues are: (i) managing pests and nutrients in ways that reduce agrochemical use; (ii) preserving the natural resource base; and (iii) protecting the genetic base for agriculture.

This review considers the aspects of sustainability of rice production involving microbial and invertebrate populations, the effects of crop intensification on these populations, the agricultural practices that utilize microorganisms and invertebrates, and the current status of germplasm collections and their biotechnological use for improving the sustainability of rice-producing environments.

## **Importance of microorganisms and invertebrates in the sustainability of rice-producing environments**

### **Maintenance of soil fertility by microorganisms and invertebrates**

From the point of view of yield sustainability, traditional wetland rice cultivation has been extremely successful. A moderate but stable yield has been maintained for thousands of years without deterioration of the environment (Bray, 1986). This is because flooding favours soil fertility and rice production by: (i) bringing soil pH near to neutral; (ii) increasing the availability of nutrients, especially phosphorous and iron; (iii) depressing soil organic matter decomposition and thus, maintaining soil nitrogen fertility;

(iv) favouring nitrogen fixation; (v) depressing outbreaks of soil-borne diseases; (vi) supplying nutrients from irrigation water; (vii) depressing weed growth, especially those of the C-4 type; and (viii) preventing water percolation and soil erosion (Watanabe *et al.*, 1988).

There is, however, no assurance that, in the long-term, crop intensification will not affect wetland soil fertility. Research on rice nutrition has shown that, at usual levels of inorganic fertilizer applied to ricefields, most nitrogen absorbed by the plant originates from the soil, where it is released by the turnover of a microbial biomass representing only a small percentage of total soil nitrogen (Watanabe *et al.*, 1988). Crop residues, rhizosphere exudates, and photosynthetic aquatic biomass (algae and aquatic plants) contribute nutrients that allow microbial biomass replenishment. Crop residues are incorporated at the beginning of the cropping season while nutrients accumulating in the photosynthetic aquatic biomass (including biologically fixed atmospheric nitrogen) are continuously recycled and reincorporated into the soil by zooplankton and the soil fauna, which are therefore key components of ricefield fertility (Roger and Kurihara, 1988). Some of the inputs allowing the replenishment of microbial biomass have been quantified, but a comprehensive understanding of the mechanisms involved in this aspect of nitrogen cycling is still to be developed. It is important to understand and predict how factors associated with crop intensification (e.g. agrochemicals) may affect the soil microbial biomass, directly or indirectly, by decreasing the productivity of the photosynthetic aquatic biomass and the populations of invertebrates responsible for recycling soil nutrients.

#### **Yield losses caused by microbial and invertebrate pests of rice**

Of the approximately 100 insect species and 74 diseases and physiological disorders associated with rice (Teng, 1990), 30 insects and 16 diseases are considered economically important (Riessig *et al.*, 1986). Table 10.1 presents a summary of estimates of yield loss due to pests and diseases.

The important insect pests of wetland rice are brown planthopper, leaf folder, stem borer, and green leafhopper (a vector of tungro virus). Important but localized losses have been attributed to rice bug (*Leptocorisa* species), gall midge larvae (*Pachytiplosis oryzae*), rice hispa (*Di cladispa armigera*), and armyworm (*Mythimna separata*) (Teng, 1986, 1990). Scarce information exists on losses caused by nematodes. Important diseases are tungro virus, sheath blight, bacterial blight, and blast (on susceptible varieties). In recent years, tungro has become a major problem in many tropical areas because of its potential to cause total loss and the lack of corrective measures once its symptoms are observed. Few data are available on yield losses caused by other diseases; but generally, under favourable conditions, most pathogens have the potential to cause severe losses (Teng *et al.*, 1990).

**Table 10.1.** Estimates of losses due to rice pests (adapted from Teng, 1990).

Agent	% Loss	Location	Reference*
<b>Insect pests</b>			
All insects	24%	Asia	(Ahrens <i>et al.</i> , 1982)
	35–44%		(Pathak and Dhaliwal, 1981)
	35%	India	(Way, 1976)
	16–30%	Philippines	(Way, 1976)
	6%	Bangladesh	(Alam, 1961)
	10–20%	Sri Lanka	(Fernando, 1966)
Rice stem borers	30–70%	Bangladesh <sup>†</sup>	(Alam <i>et al.</i> , 1972)
	3–20%	Bangladesh <sup>†</sup>	(Alam, 1961)
	3–95%	India	(Chose <i>et al.</i> , 1960)
	Up to 95%	Indonesia	(Soenardi, 1967)
	33%	Malaysia	(Wyatt, 1957)
Leafhoppers	50–80%	Bangladesh	(Alam, 1961)
Brown planthopper	1–33%	India	(Jeyaraj <i>et al.</i> , 1974)
Rice bugs <i>Leptocoris</i>	10%		(Pruthi, 1953)
Gall midge larvae	12–35%	India	(Reddy, 1967)
<i>Pachydiplosis oryzae</i>	50–100%	Vietnam	(Reddy, 1967)
Rice hispa	10–65%	Bangladesh	(Barr <i>et al.</i> , 1975)
<i>Dicladispa armigera</i>	Up to 50%	India	(Barr <i>et al.</i> , 1975)
Leaf-folders	Up to 50%	India	(Balasubramaniam <i>et al.</i> , 1973)
<b>Diseases</b>			
Blast	Up to 100%		(Teng <i>et al.</i> , 1990)
	5–10%	India	(Padmanabhan, 1965)
	3%	Japan	(Teng, 1990)
	8–14%	China	(Teng, 1986)
Brown spot	80%	India	(Padmanabhan, 1973)
	14–41%	India	(Vidhyasekaran and Ramados, 1973)
Sheath blight	9–13%	China	(Teng, 1986)
Tungro virus	100%	Indonesia	(Chang <i>et al.</i> , 1985)
	40–60%	Bangladesh	(Reddy, 1973)
	50%	Thailand	(Wathanakul and Weerapat, 1969)
	30%	Philippines	(Teng, 1990)
Bacterial blight	Up to 60%	India	(Srivastava, 1972)
	5–6%	China	(Teng, 1986)
Stem rot	5–10%	India	(Chauhan <i>et al.</i> , 1968)
	5–6%	China	(Teng, 1986)

\*Bibliographic details of references are listed in Teng (1990); <sup>†</sup>outbreak; <sup>‡</sup>chronic.

The generalized crop loss figures most commonly cited are those by Cramer (1967), who concluded that more of the rice potential production is lost due to pests (55%) than is harvested (45%). He estimated that the percentage of the potential harvest lost due to pests was 34% due to insects, 10% to diseases, and 11% to weeds. Although these figures appear to be high,

Teng (1990) found that other authors have felt that there is no sound evidence to the contrary and that these values may be underestimations of actual losses in some years. Other generalized estimates for losses caused by insects of tropical rice are 35–44% (Pathak and Dhaliwal, 1981), 24% in East and South-East Asia (Ahrens *et al.*, 1982), 35% in India, and 16–30% in the Philippines (Way, 1976).

### **Vector-borne diseases**

Wetland rice culture and irrigation schemes in tropical and subtropical regions create ecological conditions favourable to the propagation of vector-borne diseases. The most important of these are malaria, schistosomiasis, and Japanese encephalitis, whose vectors require an aquatic environment. The invertebrate vectors of human diseases in rice-growing environments are basically mosquitoes and aquatic snails.

The reproduction of mosquitoes in ricefields is affected by plant height, water depth, soil and other environmental conditions, and cultural practices. Generally, larval populations are low after transplanting, peak a few weeks later, and decline as the plants reach a height of 60–100 cm. Mosquito reproduction in ricefields ranges from 2 to 20  $m^{-2} day^{-1}$  (Roger and Bhuiyan, 1990).

Aquatic snails are very common in ricefields where they can develop large populations, especially at the beginning of the cropping season when organic manure is applied. Populations up to 1000  $m^{-2}$  have been observed in Philippine ricefields. Behavioural experiments showed that snails having to choose between various soils were most often attracted (75%) to rice.

## **Effects of crop intensification on microbial and invertebrate populations**

### **General effects of crop intensification on biodiversity in ricefields**

Traditional ricefields, some of which have been cultivated for several hundred years, may be considered as climax communities. Modern technologies, which utilize fertilizer-responsive varieties, fertilizers, pesticides, and optimum water and crop management practices, have tremendously increased yields and production but have, indeed, caused profound modifications to traditional rice-growing environments.

In general, a disturbance to a stabilized ecosystem reduces the number of species while provoking 'blooms' of certain others; such effects have been observed in ricefields (Roger and Kurihara, 1988). However, quantitative knowledge of the long-term effects of crop intensification on species diversity

**Table 10.2.** Summary of quantitative records of species/taxa in wetland ricefields.

1. Number of species recorded by Heckman in 1975 in a 1-year study of a single field in north-eastern Thailand (six samplings)

Sarcodina	31	Cyanobacteria	11
Ciliata	83	Algae	166
Rotifers	50	Pteridophyta	3
Platyhelminths	7	Monocotyledonae	25
Nematoda	7	Dicotyledonae	10
Annelida	11	Pisces	18
Mollusca	12	Amphibia/Reptilia	10
Arthropoda	146		
		Total	590

2. Number of species/taxa of aquatic invertebrates, excluding protozoa, recorded by different authors

● Heckman (1979) (species), one traditional field, 1-year study (Thailand)	183
● Lim (1980) (taxa), 2-year study of pesticide application (Malaysia)	39
● Takahashi <i>et al.</i> (1982) (taxa) four fields, single samplings (California)	10–21
● International Rice Research Institute (1985) and Roger <i>et al.</i> (1985) (species) single samplings in 18 fields with pesticide applied (Philippines and India)	2–26

3. Records of arthropod species in ricefields over one crop cycle

● Kobayashi <i>et al.</i> (1973): study in 1954–55 of several fields by net sweeping (Shikoku, Japan)	450
● Heong <i>et al.</i> (unpubl. data): study in 1989 of five ricefields by suction (Philippines):	
Fields considered separately:	146, 125, 116, 92, 87
Five fields combined	240

is extremely scarce. The only reference on the species abundance in traditional ricefields is a 1975 study by Heckman (1979) in Thailand, where 590 species (excluding fungi) were recorded in one field within 1 year (Table 10.2). Few records of aquatic invertebrates can be compared with Heckman's record of 183 species (Table 10.2). In a 2-year study of pesticide applications on Malaysian ricefields, Lim (1980) recorded 39 taxa of aquatic invertebrates. Single sampling by Takahashi *et al.* (1982) in four Californian ricefields recorded 10–21 taxa. In 18 sites in the Philippines and India, the highest number of aquatic invertebrate taxa recorded by single sampling at one site was 26, and the lowest 2 (Roger *et al.*, 1987). Similarly, records of numbers of arthropod species in Japanese ricefields estimated in 1954–1955 by net sweeping (Kobayashi *et al.*, 1973) seem to indicate a higher biodiversity than

in recent data collected by Heong *et al.* (unpublished data) in five fields in the Philippines using the suction method (Table 10.2). All the above data were obtained using different sampling methods and time frames. The marked decrease of values recorded since 1975 might probably be taken as a rough indication of a decrease in total number of species after crop intensification; however, this does not demonstrate the generally accepted concept that crop intensification decreases biodiversity in ricefields.

Crop intensification has reduced the number of edible species traditionally harvested from ricefields. Heckman (1979) reported that one vegetable and 16 edible animal species (snail, prawn, crab, large water bug, fish and frog) were collected in a single ricefield within 1 year. Such a diversity is not common anymore, and pesticides may have rendered these edible species unfit for human consumption.

Agrochemical use, besides increasing rice yield, may also cause uncontrolled growth of single species that might, directly or indirectly, have detrimental effects. One of those effects is the outbreak of pests (Heinrichs, 1988) and other organisms that may affect the fertility- or health-related aspects of the ecosystem, for example: (i) blooms of unicellular algae, observed after fertilizer application, which cause nitrogen losses by volatilization; (ii) proliferation of ostracods and chironomid larvae, observed after insecticide application, which inhibits the development of efficient nitrogen-fixing blue-green cyanobacterial blooms; and (iii) proliferation of snails or mosquito larvae that may occur after insecticide application and favour vector-borne diseases (Roger and Kurihara, 1988).

### Effects on soil and water microbial populations

Most of the information on the impacts of crop intensification on the ricefield microflora concerns pesticide use that may: (i) alter activities related to soil fertility; and (ii) reduce pesticide efficiency because of shifts in microbial populations toward organisms more efficient in their degradation. More than 200 papers, reviewed by Roger (1990), have been published on this topic, but more than half of the studies are short-term laboratory experiments in test-tubes or flasks that cannot be extrapolated to field conditions. Field and long-term laboratory studies on soil with pesticide levels near the recommended field dose allow us to draw the following conclusions.

Pesticides have three major effects on ricefield algae: (i) a selective toxicity that affects preferentially green algae and thus promotes cyanobacterial growth; (ii) a short-term promoting effect of insecticides on microalgae caused by a temporary decrease of invertebrates that graze on algae; (iii) a selective effect of insecticides on the cyanobacterial flora by causing a recruitment of grazers which results in the dominance of strains forming mucilaginous macrocolonies (e.g. *Nostoc*) resistant to grazing.

Field and laboratory studies showed that pesticides applied to soil at the recommended rates and intervals had either no effect on microbial populations or their activities, or had an effect that was followed by recovery after 1–3 weeks. Herbicides seem to have more short-term negative effects on the soil microflora than insecticides. A few studies indicate that repeated applications of the same pesticide may cause its rapid inactivation because of the enhanced growth of related specific decomposing microorganisms. This was observed in gamma-BHC, diazinon, aldicarb, and nitrophenols, but not in carbofuran and benthocarb. Repeated application of a pesticide may also change the metabolic pattern of its decomposition. In the case of benthocarb such a change produced a very phytotoxic compound (Moon and Kuwatsuka, 1984).

Because of the lack of field studies over several crop cycles, there is no information on the long-term effects of pesticide use on the wetland rice soil microflora. No method is yet available to quantify the biodiversity of the soil microflora.

### Effects on invertebrate populations

Studies of the effects of pesticides on floodwater populations show that insecticides are usually the most active compounds. Their application usually causes a general decrease in floodwater invertebrates, followed by the proliferation of primary consumers, notably ostracods, chironomid and mosquito larvae, and molluscs (Ishibashi and Ito, 1981; Roger and Kurihara, 1988), while populations of predators such as odonate larvae are reduced (Takamura and Yasuno, 1986). The rapid recovery of ostracods after pesticide application results from their resistance to pesticides and the large number of eggs they produce parthenogenetically (Lim and Wong, 1986).

Nematodes and oligochaetes are probably the only soil invertebrates studied in wetland ricefields. Usually, the specific abundance of parasitic nematodes is higher in wetlands than in uplands, but apparently this results from submersion rather than from higher agrochemical use in wetlands (J.C. Prot, personal communication). Benthocarb had no marked effect on the number of nematode species and their average populations during the crop cycle (Ishibashi and Ito, 1981). Studies at the International Rice Research Institute showed a 70% reduction in soil oligochaete populations when the amount of Furadan applied was increased from 0.1 to 1.5 kg a.i. ha<sup>-1</sup> (I. Simpson, personal communication).

### Effects on rice pests

The effects of new rice technologies on the carrying capacity of the ecosystem for insect pests were summarized by Heinrichs (1988). The availability of



short-duration varieties and irrigation water has made rice cultivation throughout the year possible, thus eliminating fallow periods that often depress insect pests. Stable water supply has favoured aquatic pests such as the caseworm *Nymphula depunctalis* (Heinrichs and Viajante, 1987). Increased nitrogen fertilizer use on responsive new varieties has favoured the brown planthopper (Denno and Roderick, 1990). In general, BPH survive better, moult into larger adults, and are more fecund if they develop on nitrogen-rich host plants. The increased yield potential of modern varieties has also resulted in the misconception by farmers that greater returns will arise from pesticide application. Many agricultural authorities have thus subsidized and encouraged insecticide use. This in turn resulted in pesticide misuse, accelerated development of resistance in pests, destruction of natural control, and pest resurgence and outbreaks.

The effects of rice production intensification on microbial pests have been summarized by Teng (1990). Crop intensification has generally resulted in increased prevalence, incidence, and severity of diseases caused by bacteria, viruses, and fungi. Bacterial blight (*Xanthomonas campestris* pv. *oryzae*) and sheath blight (*Rhizoctonia solani*) are directly attributable to cultural conditions of the modern high-yielding rice varieties which are grown with nitrogen fertilizers in large homogeneous areas. However, improved fertility associated with crop intensification has also resulted in the decrease of diseases such as brown spot (*Bipolaris oryzae*). In areas with inefficient irrigation schemes, growing several crops a year has resulted in large areas with asynchronously planted rice, which is known to favour the devastating tungro virus. Disease epidemics cause instability in rice production over time because of the pathogen's ability to overcome resistance incorporated into the rice varieties.

There is new evidence that crop intensification has no significant effect on the diversity of pathogen species in tropical rice. A study of 90 fields in the Philippines (Elazegui *et al.*, 1990) showed that the number of pesticide and nitrogen fertilizer applications had no effect on the average number of pathogenic species encountered in the fields. However, transplanted rice was richer in pathogenic species and more diverse than directly seeded rice. A denser plant population might be less conducive for pathogen dispersal within a field.

### Effects on vector-borne diseases

In traditional ricefields, although many vectors exist in the ecosystem, competition and predator pressure by fish and aquatic insects limit the productivity of any one vector. Ricefields contain a variety of insect predators of mosquito larvae such as backswimmers, gerrids, etc. (Hemiptera, Notonectidae), dragonfly and damselfly nymphs (Odonata), and adult and larval predacious water beetles (Coleoptera, Dytiscidae) (Service, 1977). Predator fauna vary

according to rice cultivars, plant height, and water management (Mather and Trinh Ton That, 1984).

In rice monoculture, with a less diverse fauna and without control measures, the productivity of some vectors may be very high. Insecticides used to control rice pests and vectors may create secondary problems. The three major effects of insecticides (Roger and Bhuyian, 1990) are: (i) the temporary decrease in vector incidence since many agricultural insecticides are non-specific and affect some vectors, extensive agricultural insecticide use probably explaining the marked reduction of malaria and Japanese encephalitis in Japan after 1945; (ii) the resurgence of resistant strains, 50 malaria vectors resistant to one or more pesticides were recorded in the world in 1987; and (iii) the adverse effects on the natural predators and competitors of vectors, causing blooming of mosquito larvae, and molluscs which are usually not affected by most rice pesticides and which multiply because of reduced predation or competition for food.

## **Use of microbial and invertebrate biodiversity to enhance agricultural sustainability of wetland soils**

### **Microbial management of wetland rice soils**

The microbial management of wetland soils was reviewed by Roger *et al.* (1991). Using biological nitrogen fixation as an alternative or supplementary nitrogen source for rice has been the major approach. Whereas nitrogen-fixing green manures (*Azolla* and legumes) have been used for centuries in some rice-growing areas, research on nitrogen-fixing cyanobacteria and bacterial inoculants for wetland rice is relatively recent, being initiated in the early 1950s for cyanobacteria and in the 1960s for other bacteria.

Biomass estimates, nitrogen-fixation measurements, and inoculation experiments indicate that cyanobacteria, as an additional nitrogen source for rice, have a potential of 20–30 kg N ha<sup>-1</sup> crop<sup>-1</sup> which may translate to a yield increase of 200–350 kg ha<sup>-1</sup>. Recent data show that cyanobacteria are ubiquitous in rice soils and that foreign strains usually do not become established in the field. Thus, the principle of cyanobacterial inoculation should be reconsidered and more attention should be paid to promoting indigenous strains.

Reported effects of the bacterial inoculation of rice have been inconsistent. Most strains tested have been nitrogen-fixing forms, but there was no clear evidence that promotion of rice growth and nitrogen uptake was due to increased biological atmospheric nitrogen fixation. Therefore, several authors refer to the production of plant growth regulators to explain the beneficial effect of bacterial inoculation. No experiment has yet supported

this hypothesis. The few data on strain establishment show that, in most cases, inoculated strains do not multiply. Given the current status of our knowledge on the bacterial inoculation of rice, no positive conclusion can be drawn as to its potential.

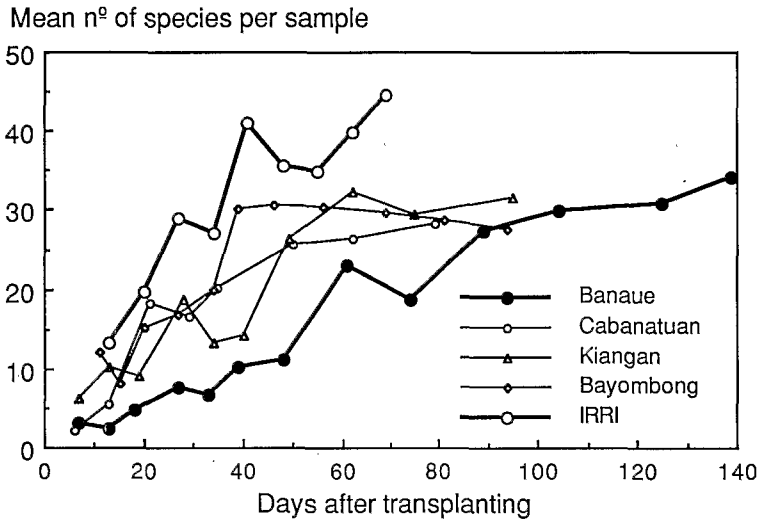
There are several reports on the existence of varietal differences in the ability to support associative biological nitrogen fixation (*N<sub>2</sub>* character). The idea of breeding varieties with higher nitrogen-fixing potential (*N<sub>2</sub>*) is attractive since it would enhance biological nitrogen fixation without additional cultural practices. This promising approach is still limited by the lack of an efficient screening method.

### Use of biodiversity to control insect pests

Natural parasitoids, predators, and pathogens that attack insect pests are abundant in ricefields. Outbreaks occur when the equilibrium is disrupted and the full reproductive capacities of pest species are released. Thus, enhancing the action of natural control agents is of paramount importance in an integrated pest management (IPM) programme. The various aspects of IPM in Asia were reviewed by Teng and Heong (1988).

Pesticides can markedly affect the natural enemy fauna and should be used judiciously. In a study comparing 330 crops in insecticide-treated and untreated fields, only 50% of the fields showed measurable yield losses due to pests (Litsinger, 1984). The removal of pesticide subsidies in Indonesia led to a drastic decrease in pesticide use with no measurable reduction in the national yield average (Kenmore, 1989). Recent data from the Philippines also showed no significant difference in average yields between farms using and not using pesticides (Elazegui *et al.*, 1990).

IPM developed from the concept of integrating control tactics into an acceptable system. These tactics include ways to maximize natural control, and the use of resistant varieties and chemicals only when necessary. It is sometimes defined as the farmers' 'best mix' of control tactics based on crop yield, profit, and safety (Kenmore *et al.*, 1985). Since IPM advocates the conservation of natural enemy populations in ricefields, more basic knowledge on population dynamics and trophic linkages is needed. Increasing general biodiversity *per se* may not be sufficient to promote ecosystem stability and sustain low-pest situations. A recent study of arthropod diversity in Philippine ricefields has confirmed this hypothesis. Of the five locations where samples were collected, the International Rice Research Institute experimental farm had significantly higher biodiversity (Fig. 10.1), but the pest population was also significantly higher (Table 10.3). The lowest biodiversity and phytophage populations were recorded in traditional rice terraces.



**Figure 10.1.** Dynamics of arthropod diversity during a crop cycle in five ricefields in the Philippines. (Banaue: rice terrace where rice has been grown without agrochemicals for centuries. Cabanatuan, Kiangang, Bayombong: farmers' fields with agrochemical use. International Rice Research Institute (IRRI): Experimental plot on the IRRI farm.)

### Use of biodiversity to control rice diseases

Natural control is not as effective with pathogens as with insect pests, but there is evidence that antagonistic bacteria can control some of the agents of rice diseases. Rosales *et al.* (1968) found that 60% of 139 ricefield bacterial isolates inhibited the *in vitro* growth of *Fusarium moniliforme* (agent of bakanae disease). Disease control by seed treatment of 18 bacterial isolates tested in artificially infested nurseries ranged from 70 to 96%. Mew and Rosales (1986) reported reductions in sheath blight severity by bacterization with *Pseudomonas* and *Bacillus* strains. These methods are not currently used in the field.

Spatial and temporal diversity of rice is a well-known strategy for disease control. Blast has been controlled by using variety mixtures (Bonman *et al.*, 1986) and one control of tungro is by rotation of host genes conferring resistance (Manwan *et al.*, 1987). The principle for using host genetic diversity to control pathogens depends on an understanding of the population genetics of the pathogen and the frequency of virulence genes in different ecosystems. While much of the theory has been developed, few empirical data yet exist in the tropics to extend the concept to pathogens other than blast and tungro.

**Table 10.3.** Arthropod community structure in five ricefields in the Philippines.\*

Sampling site	International Rice Research Institute	Cabanatuan	Bayombong	Kiangan	Banaue
Nature	Experimental farm	Farmer's fields		Rice terrace	
Species number	31	16	20	17	8
Abundant species number	13	5	11	10	6
% Contribution	82	80	60	73	59
Evenness	0.58	0.52	0.62	0.70	0.86
Total number × 1000	22	15	8	11	6
% Phytophages	64	45	43	45	57
% Predators	26	53	52	51	35

\*Average values per sampling; from Heong et al. (unpublished).

### Use of biodiversity to control vector-borne diseases

The biological control of vectors basically has two major approaches: (i) maintaining species diversity and thus conserving natural predators; and (ii) introducing new predators, competitors, parasites, or diseases of vectors.

Most of the information on the conservation of natural predators of vectors refers to mosquitoes (Roger and Bhuiyan, 1990). Because of predators such as fish, Odonata, Notonectidae, and Discidae, the survival percentage of mosquito larvae in ricefields from the first-instar through the pupal stage varies from 2 to 5%. Spiders also reduce the number of adult mosquitoes. Despite the very high predation on larvae, there may still be large numbers of adults emerging and constituting a nuisance or disease hazard. If natural predators are destroyed, emerging mosquitoes are likely to be more numerous. Even if it is not envisaged that predators will be used as control agents, cultural practices favouring their existence should be encouraged (Mather and Trinh Ton That, 1984).

Numerous competitor and predator species have been tested to control vectors. The most promising method for mosquito control is to stock food fish in and around ricefields. It reduces vector and weed incidence, increases rice yields, partly because of the fish excreta, and produces fish food (Self, 1987). In the Philippines, the combined culture of larvivorous *Tilapia* and common carp in ricefields, with supplemental feeding, produced about 700 kg fish ha<sup>-1</sup> year<sup>-1</sup> (Petr, 1987). However, experience in the efficiency of introduced larvivorous exotic fish has been varied (Roger and Bhuiyan, 1990). The main constraints to ricefield fisheries and vector control by fish are the toxicity of agrochemicals, especially pesticides, and unreliable water supply.

The use of competitors is a strategy that seems to have been restricted to snail vectors. Large snails such as *Marisa* and *Thiara* were successfully introduced and supplanted schistosome vector snails in ponds and canals in several countries, but some of these large snails feed on transplanted rice seedlings (Roger and Bhuiyan, 1990).

Microbial agents with a potential to control mosquitoes include viruses, bacteria, and fungi. *Bacillus thuringiensis* serotype H-14 and several virulent strains of *Bacillus sphaericus* (Dame et al., 1988) provide selective control of mosquito larvae, while causing relatively little harm to most of the predators of vectors and agricultural pests. Currently, only *B. thuringiensis* is used and commercialized.

Probably, the least attention to date has been given to insect predators. Their taxonomy and ecology need to be studied before their possible use in integrated pest control can be assessed and ways of multiplying them evaluated (Schaefer and Meisch, 1988).

## Preservation of microorganism biodiversity in germplasm and potential of biotechnologies for their utilization

While more than 85 000 accessions of rice are kept in the International Rice Research Institute's rice germplasm bank, other components of the ecosystem preserved in a living state include a few hundred nitrogen-fixing organisms (*Azolla*, cyanobacteria, bacteria) with potential for use as bio-fertilizers, and a few hundred strains of rice pathogens isolated from the Philippines. There is no collection of invertebrate germplasm.

### Improved strains of nitrogen-fixing agents

In view of the rapid progress in genetic engineering, one can speculate on the possibilities of selecting or designing efficient strains of nitrogen-fixing organisms.

Several authors have selected cyanobacteria with high nitrogen-fixing activities. A nitrogenase-depressed *Anabaena* mutant that excretes ammonium ions into the medium was found to provide nitrogen to rice in a nitrogen-free gnotobiotic culture more efficiently than the parent strain (Latorre *et al.*, 1986). But in both cases, strains could not establish themselves in the soil, which is consistent with the observation that no inoculation experiment has yet reported the establishment of foreign cyanobacterial strains in soils (Roger *et al.*, 1991). Significant progress has been made on cyanobacterial genetics, and 'super' nitrogen-fixing cyanobacteria can be selected or probably designed and grown *in vitro*, but the characteristics that will enable them to survive, develop, and fix nitrogen as programmed *in situ* are still unknown.

*Azolla* collections have been used to screen varieties adapted to specific environments, while some efficient strains have been adopted for practical use. These collections have also been used to achieve the exchanges of cyanobacterial symbionts between species, and for breeding improved *Azolla* hybrids (Roger *et al.*, 1991).

### Characterization of inter- and intraspecific diversity of rice pathogens

Sustainable rice ecosystems need to provide, *inter alia*, economic stability to the farmer as demonstrated by stable rice yields. One of the main causes of instability are the sporadic epidemics caused by subpopulations of plant pathogens increasing in frequency relative to the prevailing rice genotype. This results in the so-called 'boom-and-bust' cycles of varietal resistance breakdown (Teng, 1990). Understanding the coevolutionary processes between pathogen populations and rice requires the monitoring of pathogen species at the community level, which was constrained historically by the lack of rapid and accurate methods of characterization. Pathogen identification

commonly requires time-consuming axenization of the pathogen to fulfil Koch's postulates. Tools such as polyclonal and monoclonal antibodies have reduced the time needed for identification, but antibodies have only been developed against a few rice pathogen species and this is an area which requires greater effort.

Within the same species, pathogen populations also exhibit much genetic variability on the same rice genotype. The traditional method of studying such variability has been to 'type' the subpopulations using a set of rice differential genotypes representing a range of susceptibilities to the pathogen. With modern molecular markers and selected serological techniques, the tedious and complicated process of race typing has been greatly simplified and made more reliable. Intraspecific diversity of *Xanthomonas campestris* has been recently studied at the DNA level through the detection of restriction fragment length polymorphisms (RFLP) (Raymundo *et al.*, 1990). DNA probes were used to study the partitioning of variability in pathogen populations. Several putative transposable elements were also identified and used to examine the DNA profiles of a collection of strains. The results indicate a particular evolutionary relationship between pathogenic races and rice host resistance. The intraspecific diversity revealed by RFLP typing allows the selection of appropriate tester strains to identify unrecognized races and resistance genes.

## Conclusions

The beneficial and detrimental roles of many groups of microorganisms and invertebrates in sustainable rice production have been identified, and sometimes, quantified. However, knowledge on the possible long-term effects associated with the intensification of rice cultivation on these populations is limited. The study of crop intensification effects in long-term experiments should have high priority. In particular, the nitrogen fertility of wetland rice soils depends upon the turnover of a soil microbial biomass representing only a small percentage of total soil nitrogen. Therefore, a general understanding is needed of: (i) the pathways that allow microbial biomass replenishment; and (ii) the long-term effects of crop intensification on microbial and invertebrate populations involved in this replenishment.

Estimates of biodiversity in ricefields are extremely scarce; there are no irrefutable data to demonstrate the generally accepted concept that crop intensification decreases biodiversity in ricefields.

Recent data on arthropods confirm that high biodiversity is not synonymous with stability and with low pressure of insect pests. Increases in pest and vector densities may depend primarily on reduced predator diversity and the resilience and biological attributes of a particular pest. Thus, increasing or preserving diversity *per se* does not necessarily contribute to pest stability,



but developing effective trophic linkages might. This approach might also be valid for maintaining soil fertility through microbial management, the optimization of the primary production in floodwater, and the optimization of nutrient recycling by invertebrate populations.

Numerous methods using microorganisms and invertebrates to increase soil fertility (especially through biological nitrogen fixation) and to control pests and diseases have been tested. But the success of these methods is limited and their adoption is almost negligible. This situation will probably remain unchanged as long as the methods designed continue to be based on an extremely restricted knowledge of biodiversity, community structure, and trophic relationships at the ecosystem level.

However, current knowledge shows that there is potential in designing methods that use microbial and invertebrate populations to sustain a management system that reduces and optimizes agrochemical use.

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## Discussion

**Greenland** Could Dr Roger comment on the biodiversity among cyanobacteria in rice paddy fields? Does it relate to the magnitude of biological nitrogen fixation and to the long-term fertility of the paddy soils?

**Roger:** Our current state of knowledge, based on over 800 papers published on this topic, is that it is not the absence of cyanobacteria which is the cause of the low nitrogen fixation, but that the limiting factors are grazing, a lack of phosphorous, and inhibitory applications of nitrogen fertilizers. Cyanobacteria are now known to be ubiquitous in rice fields and only alleviation of the limiting factors is required. It is important to stress that there is currently no satisfactory taxonomy of cyanobacteria. We lack methods to enable field workers to identify strains. Much published inoculation work does not include records of the strains used and has made it impossible to follow the fate of the inoculated strains. The new biomolecular tools now available could change this.