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### **Paddy soils and water scarcity**

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# Carbon sequestration in paddy ecosystems in subtropical China

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## Abstract

Carbon (C) sequestration in paddy soils was studied in 4 selected landscape units as representatives for the lowland (LL), low-hill (LH), high-hill (HH) and karst-mountain (KM) areas in subtropical China. The mean values for organic C content in paddy soils (0-20cm) in the landscape unit varied from 16.0 to 27.7 g/kg, which were remarkably larger than those for soils under arable cropping and orchard, and even under woodland except in the KM landscape unit. In the LH landscape unit, the mean organic C content in paddy soils increased by 1.67 times ( $P < 0.01$ ) in the period of 1979-2003. This increase was concordant with the prolonged increase (since 1950s) in rice productivity in the region. It is concluded that paddy ecosystems in subtropical China had the ability to sequester organic C in amounts larger than those in other ecosystems. As these landscape units represent the real situations for paddy ecosystems under farmers' practices for rice production, data from this study confirm that the trend of continuing organic C sequestration in paddy soils occurred in subtropical China.

## Key Words

Carbon stock, paddy soil, landscape unit, subtropical China.

## Introduction

Changes in soil organic C by agriculture affect terrestrial C stock, which is a twice as large as the atmospheric C stock and the associated global changes (IPCC 2007). Rice cultivation globally covers a total area of about 153 million ha (FAOSTAT database) and has been proposed to have a great potential in sequestering atmospheric CO<sub>2</sub> (Lal 2004; IPCC 2007). This paper was aimed to understand the C sequestration capability of paddy ecosystems in a landscape scale in subtropical China.

## Materials and methodology

Four landscape units were selected as the representatives of the land types of lowland (LL; with an elevation of 35-45 m), low-hill (LH, 81-122 m), high-hill (HH; 202-395), and karst-mountain (KM; 202-450 m) areas, which cover the major region of subtropical China (Table 1). They contained multiple ecosystems with native woodlands and various cropping lands including paddy, arable and orchard fields under farmers' practices, providing real information for evaluating the impacts of agriculture on soil C stock at the landscape scale. Each of the landscape units selected covered an area of 5-8 km<sup>2</sup>. For each landscape unit, samples of surface soil (0-20 cm) at 538-744 sites (about 3-4 /ha for farmlands and 1 /ha for woodland and orchards) were collected between July, 2003, and March, 2004. Each was taken as 6-10 soil cores (Ø 3.0 cm). In the same area where the LH landscape unit was selected, 347 and 115 additional samples were taken in 1979 and 1990, respectively.

Organic C content of the soils from the low hill and high hill landscape units was measured by the combustion method using an automated C/N analyzer (vario MAX, Elementar, Germany). Because soils from the LL and KM landscape units contained CaCO<sub>3</sub>, which interferes with instrumental analysis, organic C was determined by the traditional wet digestion with potassium dichromate.

## Results and Discussion

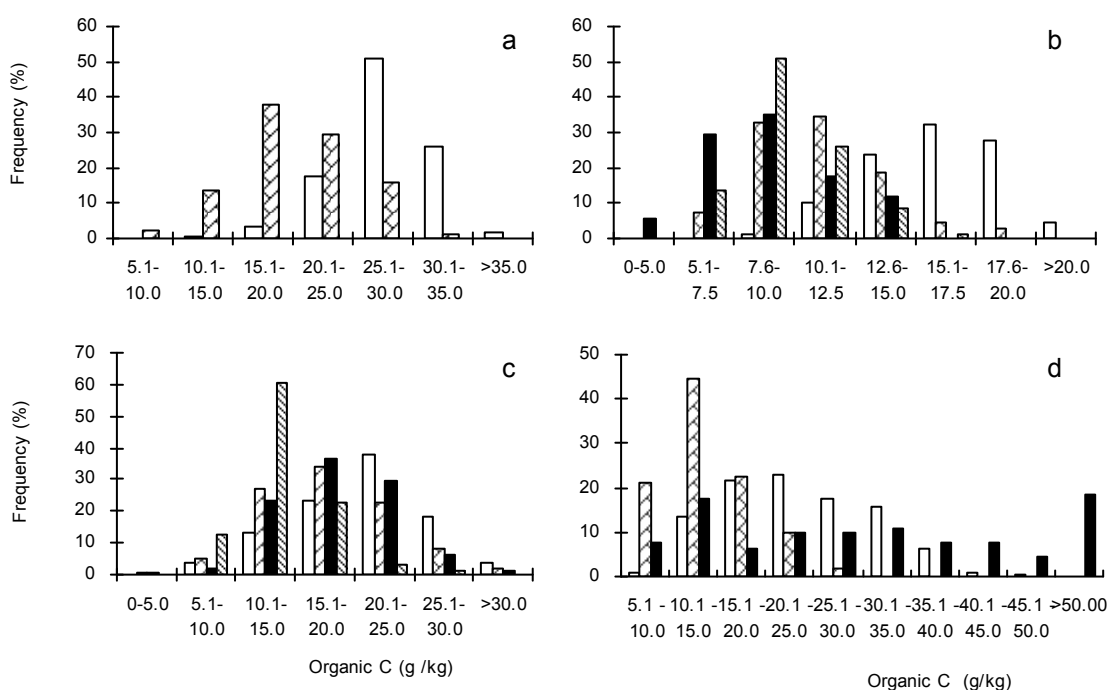
In the LL landscape unit, about 94% of paddy soils (0-20 cm) had the values of organic C content ranging from 20.1 to 35.0 g/kg, with a peak distribution (51%) occurring in the range of 25.1 to 30.0 g/kg (Figure 1a). However, the values for arable soils distributed mainly in a distinctly lower range, from 15.1 to 25.0 g C/kg.

In the LH landscape unit, the majority (84%) of paddy soils contained organic C ranging from 12.6 to 20.0 g/kg (Figure 1b). For arable and woodland soils, organic C content ranged mainly from 7.6 to 15.0 g/kg. The range for the majority of orchard soils was narrowed to 7.6 to 10.0 g C/kg.

**Table 1. Mean and coefficient of variation of soil organic C content in selected landscape units**

Landscape unit	Land use	Sample number	Organic C content		
			Mean (g /kg) <sup>A</sup>	SD	C.V. (%)
Lowland (LL)	Paddy	545	27.7 a	4.1	14.7
	Arable	82	19.9 b	4.9	24.5
Low-hill (LH)	Paddy	253	16.0 a	2.7	16.6
	Arable	70	11.2 b	2.8	25.2
	Woodland	17	8.4 b	2.3	28.0
	Orchard	198	9.5 b	2.0	21.2
High-hill (HH)	Paddy	77	21.0 a	5.4	25.9
	Arable	336	17.8 ab	5.4	30.5
	Woodland	197	18.2 ab	4.9	26.8
	Orchard	97	13.6 b	3.3	24.4
Karst-mountainous (KM)	Paddy	339	22.9 b	7.2	31.5
	Arable	313	13.9 c	4.7	33.7
	Woodland	92	35.3 a	25.2	71.5

<sup>A</sup>Letters indicate the significant difference for the mean between land uses ( $p < 0.05$ )



**Figure 1. frequency distribution of organic C content in soils in the lowland (a), low-hill (b), high-hill (c), and Karst-mountain (d) landscape units selected in subtropical China. Paddy soils (□), Arable soils (▨), Woodland soils (■), and Orchard soil (▩).**

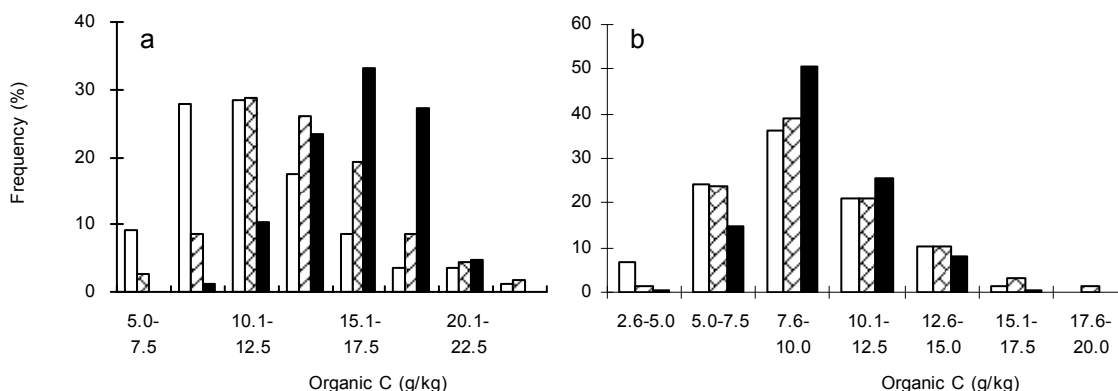
Although the majority (61%) of paddy soils contained organic C in the range of 15.1 to 25.1 g /kg in the HH landscape unit, the frequency distribution increased steadily from about 4% below 10 g C /kg to 38% within the range 20.1 to 25.0 g C /kg, then decreased (Figure 1c). The distribution of organic C content in arable and woodland soils was very similar, with 87% in the range of 10.1 to 25.0 g /kg. However, the majority of orchard soils contained organic C in a much narrower range, because 61% appeared between 10.1 and 15.0 g /kg.

In the KM landscape unit, the majority of paddy soils contained organic C in the range from 15.1 to 35.0 g /kg (Figure 1d). This range was much wider than that for the three landscape units described above. Organic C content for arable soils in this landscape unit was distributed mainly (88%) in the range of 5.1 to 20.0 g /kg, with a peak distribution (44%) appearing in the range of 10.1-15 g /kg. However, woodland soils showed a wider range of organic C values (from 5.1 to over 50.0 g /kg). For paddy and arable soils, the mean values for organic C content in the LL landscape unit were significantly larger than those in other three landscape units (LH, HH, and KM) (Table 1). However, the mean values for all of the 4 land-use types

(paddy, arable, orchard, and woodland) in the LH landscape unit were significantly smaller than those in other three landscape units (LL, HH, and KM). For woodland soils, the mean values of organic C content differed significantly in the order of KM>HH>LH.

Our data also indicated that there were large differences in the distribution frequency of organic C content in paddy soils in the different landscape types, as 20.1–35.0 g/kg for LL, 12.6–20.0 g/kg for LH, 15.1–25.0 g/kg for HH, and 15.1–35.0 g C/kg for KM (Figure 1). Both of paddy soils and arable soils in the LL landscape unit had been converted from wetland ecosystems between 1950s and 1970s. Therefore, the differences between organic C contents in these two types of soils indicated a large loss of soil organic C under arable cultivation in the area (Table 1). Clearing native vegetation such as forest and grassland in order to create land for farming usually results in a loss of soil organic C (Jenkinson 1990). However, data obtained in the present study suggest that rice cultivation seems to be an exception. This is in agreement with previous studies with historical data in subtropical China (Li and Zhao 2001) and field trials (e.g. Pampolino *et al.* 2008) in India. For all of the four landscape units investigated, paddy soils showed significant higher organic C content, compared to those in soils under arable and orchard management, and also under woodlands, with the exception of woodland soils in the KM landscape unit.

In paddy soils sampled in 1979 from the low-hill area from where the LH landscape unit was selected, about 56% had organic C contents ranging from 7.6 to 12.5 g/kg. As organic C content increased, the distribution frequency of soils decreased sharply (Figure 2a). By 1990, organic C content of paddy soils in this landscape unit mainly distributed in the range of 10.1–17.5 g/kg, and then moved to a significantly higher range (15.0 and 17.5 g C/kg) by 2003. Therefore, the mean value of organic C content in these soils ( $16.0 \pm 2.7$  g/kg) was 17% ( $p < 0.01$ ) larger than that ( $13.7 \pm 3.0$  g/kg) in 1990, or 60% larger than that (10.0 g/kg) in 1979, respectively. In the same landscape unit, the distribution frequency of organic C in arable soils sampled in 1990 and 2003 was very similar to that for soils sampled in 1979 in the low-hill area, with a peak distribution (about 40%) appearing the range of 7.6–10.0 g/kg (Figure 2b), and the mean of organic C in the arable soils crossing these three sampling times were comparable. These results provide clear evidence of a temporal increase in organic C content in paddy soils at the landscape scale, suggesting that the trend for organic C sequestration by paddy soils was a real one.



**Figure 2. Temporal change (1979–2003) in the frequency distribution of organic C content in (a) paddy and (b) arable soils in the low-hill landscape unit (□1979 ▨1990 ■2003).**

Organic inputs from ground cover vegetation or external manure sources are essential for maintaining the cycling and sequestration of organic C in soil. Based on data from China Agriculture Yearbook, the mean grain yield of rice (single crop) in subtropical China increased from about 2 t/ha in the 1950s to 3.5–4.0 t/ha in 1977. because double-rice systems have been widely introduced in this region, together with a continuous increase in the application of chemical fertilizers, the mean yield was increased further to about 11 t/ha by the mid-1990s and afterwards. Assuming that the primary productivity and the amount residue of rice was proportionally related to grain yield, the amount of C input as straw and root biomass to cultivated rice soils must have increased by approximately a similar extent between 1978 and 2003. It is logical to suggest that such a large increase in organic inputs can support prolonged C sequestration by paddy soils in subtropical China.

## Conclusion

Investigations in the landscape units suggest that paddy ecosystems in subtropical China have the ability to sequester organic C in amounts that are larger than those in other ecosystems. Because these landscape units represent the real situations for the paddy ecosystems under farmers' practices for rice production, the data obtained by in this study indicate a trend of continuing organic C sequestration in paddy soils in subtropical China.

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# Changes in community structure and transcriptional activity of methanogenic archaea in a paddy field soil brought about by a water-saving practice – Estimation by PCR-DGGE and qPCR of 16S rDNA and 16S rRNA

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## Abstract

Effect of a water-saving practice on the methanogenic archaeal community in a paddy field soil of the International Rice Research Institute (IRRI) Farm was investigated by PCR-DGGE and qPCR targeting 16S rDNA and 16S rRNA. Plow-layer soil samples were periodically collected from field plots under the treatments of continuously flooding (Control) and an alternate wetting and drying (AWD) water-saving during a rice cultivation period in 2008. Both DGGE band patterns of 16S rDNA and 16S rRNA were relatively stable throughout the rice cultivation period irrespective of the treatments. Cluster analysis showed a tendency that the patterns of the AWD plots were separated from those of the Control plots. Principal component analysis and sequencing analysis of 16S rDNA indicated members of *Methanosarcinales* and *Methanocellales* mainly characterized the Control and AWD plots, respectively. Numbers of 16S rDNAs significantly fluctuated during the rice cultivation period and differed between the Control and AWD plots. Those of 16S rRNAs decreased in the AWD plots in the last half of the rice cultivation period although the fluctuations were not significant. These results suggest that the water-saving management brings about changes in both community structures and transcriptional activities of methanogenic archaea in paddy field soil.

## Key Words

Methanogenic archaea, Paddy field soil, Water-saving irrigation, PCR-DGGE, qPCR

## Introduction

Water scarcity caused by climate changes, growth of population, etc. is one of the serious problems in the world. In the International Rice Research Institute (IRRI), alternate wetting and drying (AWD) irrigation technique (Bouman *et al.* 2007) has been developed to reduce water use without any adverse effects on rice production. The AWD technique is a kind of intermittent irrigation management and enables reduction by 15-20% of irrigation water (Tabbal *et al.* 2002; Belder *et al.* 2004) and 35-45 % of annual methane emission (Hosen *et al.* unpublished data) from paddy fields, compared with a conventional continuous flooding water management. Methane is one of the greenhouse gases and paddy fields are known as a major source of atmospheric methane. Methanogenic archaea play a unique role for biological methane production in paddy fields. In relation to the reduction of methane emission by the AWD managements, it is conceivable that changes in community structure and metabolic activity of methanogenic archaea influenced the methane emission from AWD paddy fields. In the present study, therefore, the effect of AWD management on the methanogenic archaeal community in a paddy field soil was evaluated by molecular ecological techniques (PCR-DGGE and qPCR) targeting their 16S rDNA and 16S rRNA.

## Materials and methods

### *Investigated field*

The experiment was conducted at the IRRI Farm, Los Baños, Philippines (14°30'N, 121°1'E). The soil (organic C, 1.68 %; total N, 0.17 %; pH 7.0) was a clayey loam Aquandic Epiaquoll (Dobermann *et al.* 2000). Two treatments with three replication plots were chosen: continuous flooding (Control) plots and AWD water-saving plots. The Control plots were basically kept under flooded condition during the rice cultivation period. Irrigation of the AWD plots was basically carried out when soil water potential at 15 cm depth reached -20 kPa except for the early growth and heading periods of rice when the fields were kept under a flooded condition.

### Soil sampling

Soil samples were collected four times during the rice cultivation period in the wet season in 2008: 15 July (1st, 15 days after transplanting [DAT]), 12 August (2nd, 33 DAT), 8 September (3rd, 60 DAT) and 9 October (4th, 91 DAT). The 1st sampling was carried out when the both Control and AWD plots were under a flooded condition. The 2nd–4th samplings were carried out when the depth of water decreased to 0 cm (i.e. soil surface) in two of three replicated plots as it was predicted that methanogenic activities in the AWD paddy soils would reach a peak after soil submergence. Soil samples were collected from 3–13 cm depth of the plow-layer soil. In total, 600 ml of soil sample was collected from three spots in each replication and composited in a clean plastic bag. The collected soils were immediately brought back to the laboratory and mixed well in the plastic bags. The soil samples were stored in -20 °C and -80 °C freezers for DNA and RNA extraction, respectively.

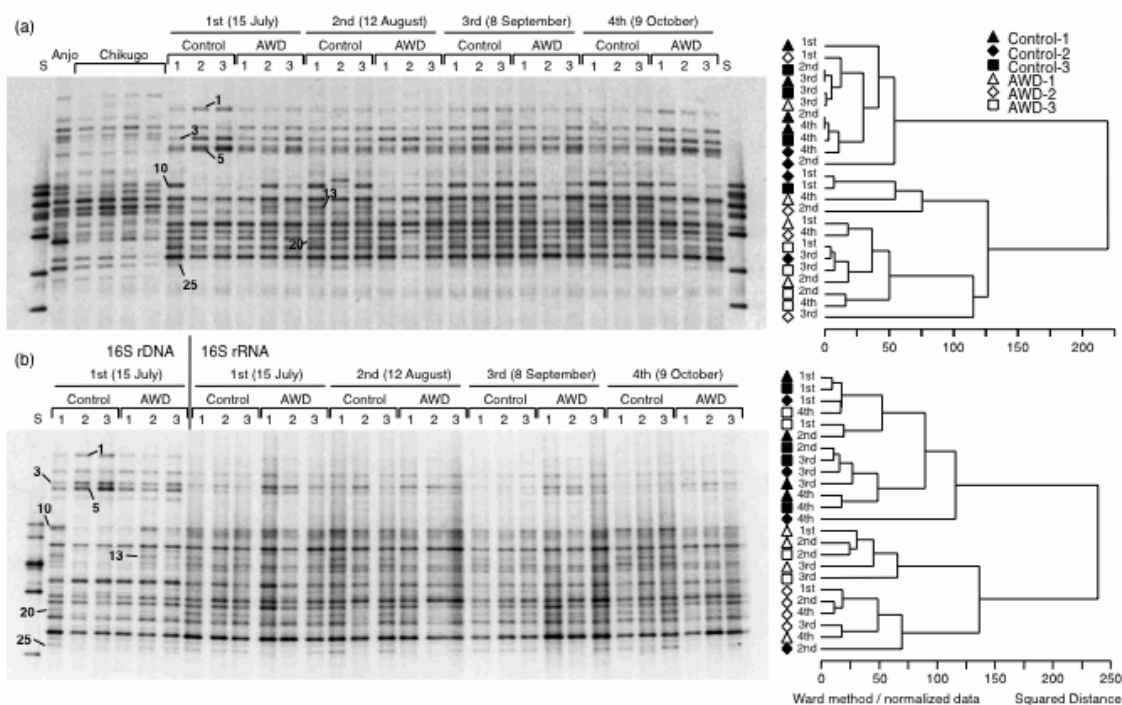
### Molecular ecological analysis of methanogenic archaeal community

DNA and RNA were separately extracted from the soil samples by the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH USA) and following the procedure described by Watanabe *et al.* (2007), respectively. cDNA was synthesized from extracted RNA by reverse transcription reaction using SYBR PrimeScript RT reagent kit (TaKaRa, Shiga, Japan). PCR-DGGE using the primers 1106F-GC/1378R and subsequent multivariate analyses (cluster analysis and principal component analysis [PCA]) were carried out, as described by Watanabe *et al.* (2006). Numbers of methanogenic archaeal 16S rDNAs and 16S rRNAs were determined by qPCR analysis using the LightCycler (Roche Diagnostics, Basel, Switzerland), as described by Watanabe *et al.* (2007). Nucleotide sequences of 16S rDNA fragments recovered from the DGGE bands were determined by the direct sequencing method as described Watanabe *et al.* (2004). Phylogenetic affiliations of the sequences were determined by the BLAST program on the DDBJ web site.

## Results

### PCR-DGGE analysis of methanogenic archaeal 16S rDNA and 16S rRNA

Figure 1 shows DGGE band patterns of methanogenic archaeal 16S rDNA and 16S rRNA retrieved from the soils in the Control and AWD plots. Twenty-seven bands were observed at different positions in the both DGGE band patterns. The numbers of bands fluctuated between 17 and 24 and 17 and 22 among the patterns of 16S rDNA and 16S rRNA, respectively. However, major bands with strong intensity were commonly observed in both DGGE band patterns irrespective of the water management.

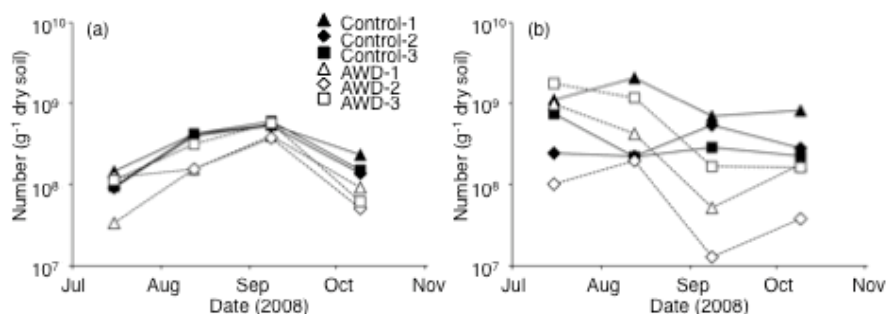


**Figure 1.** DGGE band patterns and cluster analysis of methanogenic archaeal 16S DNA (a) and 16S rRNA (b) obtained from the Control and AWD paddy field soils at IRRI. Anjo and Chikugo show the patterns obtained from Japanese paddy field soils, where a previous investigation was performed (Watanabe *et al.* 2006). S is a mixture of PCR amplicons derived from 13 strains of methanogenic archaea. The denaturant gradient range was 32 to 62 %.

Cluster analysis and PCA were carried out, based on relative intensity and mobility of the DGGE bands. Both analyses of the DGGE band patterns of 16S rDNA and 16S rRNA showed a tendency for the Control and AWD samples to cluster separately although the patterns obtained from the 1st sampling did not show uniformity. These findings suggest that the community structures and transcriptional activities of methanogenic archaea in the both Control and AWD plots changed during the rice cultivation period, but the changes were different between the Control and AWD plots. The DGGE bands characterizing the Control and AWD plots were determined from the coefficient of PCA (Figure 1; the bands 10, 13, 20 and 25 for the Control and 1, 3, and 5 for the AWD plots).

#### Quantification of methanogenic archaeal 16S rDNA and 16S rRNA

Numbers of methanogenic archaeal 16S rDNAs in the Control and AWD plots fluctuated between  $1.1 \times 10^8$  and  $5.6 \times 10^8$  and  $6.8 \times 10^7$  and  $4.5 \times 10^8$ /g dry soil, respectively (Figure 2). Those of 16S rRNAs in the Control and AWD plots ranged from  $4.4 \times 10^8$  to  $8.4 \times 10^8$  and  $7.7 \times 10^7$  to  $9.5 \times 10^8$ /g dry soil, respectively. Although the maximum numbers of 16S rDNAs were almost same between the Control and AWD plots, the numbers in the AWD plots gradually increased and rapidly decreased. The numbers of 16S rDNAs were significantly different between the sampling dates ( $P < 0.001$ ) and between the Control and AWD plots ( $P < 0.05$ ). A mutual influence between the sampling date and water management was also observed ( $P < 0.05$ ). The numbers of 16S rRNAs in the Control plots did not fluctuate throughout the rice cultivation period, while those in the AWD plots tended to decrease in the last half of the rice cultivation period although the differences were not significant. These results indicate that the AWD management repressed proliferation and metabolic activity of methanogenic archaea, compared with the Control plots. The repressions may partly contribute to the reduction of methane emission from the AWD paddy fields.



**Figure 2.** Number of methanogenic archaeal 16S rDNAs and 16S rRNAs in the Control and AWD paddy field soils during the rice cultivation period of wet season 2008 (means of each replication plot,  $n = 3$ ).

#### Phylogenetic affiliation of methanogenic archaeal 16S rDNA

In total, 26 DGGE bands were successfully sequenced from the band patterns of 16S rDNA. Those sequences were affiliated with *Methanobacterium* spp., *Methanosarcina* spp., *Methanosaeta* spp., uncultured group of ZC-I in *Methanosarcinales*, uncultured group of *Methanomicrobiales*, *Methanocellales* (formerly, Rice cluster I) and Crenarchaeota. Sequences of the DGGE bands, which characterized the Control plots (bands 10, 13, 20 and 25 in Figure 1), were closely related to members of *Methanosaeta* spp. (band 10) and the ZC-I cluster (bands 13, 20 and 25). It is known that *Methanosaeta* spp. use only acetate for methanogenesis (Garcia *et al.* 2000). The members in ZC-I cluster are enriched in the Zoige wetland of the Tibetan plateau (Zhang *et al.* 2008) and are assumed to use acetate,  $H_2/CO_2$ , methanol and trimethylamine as substrates for methanogenesis. These findings suggest that acetoclastic methanogenic archaea and methanogenesis became more dominant in the Control paddy fields during the rice cultivation period. Previous studies investigating *mcrA* genes and their transcripts in a Japanese paddy field soil also showed uncultured members of *Methanosarcinales* actively transcribed *mcrA* genes under a flooded condition (Watanabe *et al.* 2009). On the other hand, sequences characterizing the AWD plots (bands 1, 3 and 5 in Figure 1) were affiliated with members of *Methanocellales* (band 3) and uncultured Crenarchaeota (bands 1 and 5). Although all methanogenic archaea hitherto isolated are strictly anaerobic microorganisms, it has been estimated from genome information that a member of *Methanocellales* possess multiple sets of genes encoding antioxidant enzymes (Erkel *et al.* 2006). Previous study showed that *mcrA* transcripts derived from *Methanocellales* were preferentially recovered from a Japanese paddy field soil under unflooded condition (Watanabe *et al.* 2009). Therefore, these members might be relatively resistant to the oxic condition in the AWD paddy field. Members of *Methanocellales* are known as hydrogenotrophic methanogenic archaea. Population and transcription activity of methanogenic archaea increased gradually in the AWD plots,

compared with the Control plots, suggesting acetoclastic methanogenic archaea could not actively proliferate and produce methane in the AWD plots. These findings indicated that the AWD management changed the methanogenic pathway (acetate vs.  $H_2/CO_2$ ) in the paddy field soil.

## Conclusion

DGGE band patterns of methanogenic archaeal 16S rDNA and 16S rRNA obtained from the AWD paddy field soil were relatively stable through the rice cultivation period, but multivariate analyses showed a tendency for patterns differ between the Control and AWD plots. The numbers of methanogenic archaeal 16S rDNAs fluctuated during the rice cultivation period and differed between the Control and AWD plots. Those of 16S rRNAs in the AWD plots tended to decrease in the last half of the rice cultivation. Phylogenetic analysis indicated that methanogenic pathway differently changed depending on the Control and AWD plots. These results suggest that AWD water-saving management moderately brings about changes in community structure (composition and population) and transcriptional activities of methanogenic archaea and the changes partly contribute to the reduction of methane emitted from the paddy field.

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# Changes in paddy soils under transition to water-saving and diversified cropping systems

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## Abstract

Most rice (*Oryza sativa* L.) is produced on soils with a prolonged period of submergence. Soil submergence has helped sustain the productivity of continuous rice production systems. It helps maintain soil organic matter (SOM), favors input of N through biological nitrogen fixation, and enhances availability of soil P to rice. Rice will increasingly be produced within political and economic environments of less supply of irrigation water and more income opportunities from alternative crops. This will lead to changes in water management, rice cultivation practices, and cropping patterns resulting in reduced soil submergence and increased duration of soil aeration. Soil aeration alters soil biogeochemical processes, which can lead to loss of SOM, reduced supply of plant-available N and P, and reduced zinc and iron availability on high-pH soils. Soil aeration favors the formation of nitrate, which can be lost via denitrification upon soil submergence for rice cultivation. Soil drying and wetting favor increased emission of nitrous oxide and reduced emission of methane. The productivity of paddy soils, which has been sustained with ample water resources, must in the future be sustained with management interventions that more effectively use water and provide enhanced crop diversification and income generation.

## Key Words

Paddy soils, rice, water savings, crop diversification, soil organic matter, nitrogen.

## Introduction

“Paddy soils” denote soils in irrigated and rainfed lowland rice production systems with a prolonged period of submergence. About 90% of the global area of paddy soils is located in Asia. Soil submergence leads to unique biogeochemical processes, which influence ecosystem sustainability and ecosystem services such as carbon storage, nutrient cycling, and nutrient supply to crops. Soil submergence does not occur in the production of other major food crops, and the management of natural resources differs between landscapes dominated by lowland rice and other agricultural land-uses.

Rice produced on submerged soil is a major beneficiary of freshwater resources. An estimated 24% to 30% of the world’s developed freshwater resources are used for the irrigation of rice (Bouman *et al.* 2006). Much of the world’s rice is produced in countries with rapidly growing economies. With economic growth comes competing demand for use of water by industries and households rather than agriculture. Groundwater has become an important source for irrigation, particularly in South Asia, but groundwater tables are falling in many areas. Thus, there are valid concerns regarding how the reduced availability and increased price of irrigation water will affect paddy soils and rice production.

Production systems on paddy soils include one, two, or three rice crops per year. Double and triple cropping of continuous monoculture rice account for about 40% of the global rice supply. Rice is also commonly grown in rotation with other crops on paddy soils. The rice–wheat (*Triticum aestivum* L.) cropping system is common in the subtropics of South Asia and China. The rice–maize (*Zea mays* L.) cropping system is gaining importance on paddy soils across tropical and subtropical Asia in response to the increasing demand of maize for feed and biofuel.

Rice will increasingly be produced within political, economic, and social environments of less supply of irrigation water and more income opportunities from crop diversification. This could result in changes in water management, cultivation practices, and cropping patterns leading to reduced soil submergence and increased duration of soil aeration. A change from soil submergence to greater soil aeration, while increasing the efficiency of water use, can significantly affect the biogeochemical processes influencing carbon storage, nutrient cycling and supply to crops, greenhouse gas emissions, and rice productivity. In this paper, we review changes in paddy soils arising from both growing rice with less water and switching to more diversified cropping systems.

### **Effect of growing rice with less water**

Continuous rice cropping with up to three crops per year was made possible with short-duration, high-yielding rice cultivars and irrigation. Rice in Asia is traditionally established on paddy soil by transplanting, but as labor costs increase there has been a move to direct wet seeding of germinated seed. Land preparation for both transplanted and wet-seeded rice typically consists of soaking the soil followed by plowing and harrowing of saturated soil. The tillage of saturated soil — referred to as puddling — destroys soil structure, creates a soft muddy layer of 10–15 cm depth, and reduces subsequent downward movement and loss of water during rice cropping. Rice fields are typically kept submerged with 5 cm or more of water throughout the growing season until just prior to harvest. This submergence helps control weeds and increase the availability of a number of nutrients. The production of irrigated rice consequently requires considerable water because of the high water use for land preparation and the losses by seepage, percolation, and evaporation when soil is submerged (Bouman *et al.* 2006).

#### *Alternate wetting and drying of puddled soil*

The use of irrigation water for producing lowland rice on puddled paddy soils can potentially be reduced by lowering the depth of standing water and by allowing the soil surface to dry before the next application of irrigation water. The practice of withholding irrigation until several days after the disappearance of ponded water is referred to as alternate wetting and drying (AWD) (Bouman *et al.* 2007). Even without ponded water, rice roots can access the water in the subsurface soil, which remains saturated. The practice of “safe” AWD now promoted as a mature water-saving technology entails irrigation when water depth falls to a threshold depth below the soil surface. Safe AWD results in a savings of irrigation water, increased water productivity, and no decline in rice yield (Bouman *et al.* 2007).

AWD results in periodic soil aeration, but the extent and duration of soil drying when implemented at safe levels, which do not result in loss of rice yield, are unlikely to have much effect on soil organic matter (SOM) and plant availability of macronutrients in the soil. No adjustments in management practices for fertilizer N, P, and K are proposed with safe AWD. Nutrient best management practices based on the principles of site-specific nutrient management (SSNM) (Buresh 2010, IRRI 2010) are the same for rice grown with AWD and continuous soil submergence, provided AWD does not result in water stress leading to lower attainable yield. Broadcasting urea before irrigation could help ensure the movement of N into the soil, where it would be less prone to loss via ammonia volatilization (Buresh *et al.* 2008).

Sequential nitrification-denitrification is greater in soil with alternate wetting and drying than with continuous submergence (Buresh *et al.* 2008). AWD could consequently lead to a slightly greater loss of broadcast fertilizer N and soil N by nitrification-denitrification, but this loss is expected to decrease with increasing age of the rice crop due to increased competition of rice with microorganisms for ammonium before it can be nitrified and for nitrate before it can be denitrified (Buresh *et al.* 1993).

Erratic rainfall in rainfed rice ecosystems and periodic unavailability of irrigation water in irrigated rice ecosystems can prolong the duration of soil drying, resulting in soil water deficit, leading to a loss in rice yield. As a general principle, as soil drying becomes more prolonged and severe, the availability of soil P to rice tends to decrease and the availability of zinc in acid soils tends to increase (Dobermann and Fairhurst 2000). Fertilizer rates should be adjusted to the anticipated water-limited grain yield of rice (Haefele and Bouman 2009).

#### *Prolonged soil aeration of nonpuddled soils*

Another approach to growing rice with reduced water use is to grow rice as an upland crop — like wheat or maize — on nonpuddled, nonsaturated soil without ponded water. When rainfall is insufficient to maintain soil water content above a threshold between field capacity and wilting point, irrigation water can be applied to bring soil water content in the root zone to field capacity. This practice of rice cultivation under conditions of relatively severe shortages of irrigation water is referred to as “aerobic rice” (Bouman *et al.* 2007). The paddy soil remains aerated (aerobic) throughout the rice-growing cycle.

Rice production on aerobic soils often uses conventional full tillage of aerated soil for seedbed preparation, initial weed control, and crop establishment by direct dry seeding of nongerminated seed (Bouman *et al.* 2007). Alternatively, resource-conserving technologies are developed to grow rice using reduced or zero tillage and establishment by drill seeding or transplanting (Ladha *et al.* 2009).

The submergence of rice soils helps maintain SOM, even with intensive rice cropping and removal of crop residues (Pampolino *et al.* 2008). This maintenance of SOM ensures that C remains sequestered in the soil. Soil submergence also promotes biological nitrogen fixation (BNF) (Buresh *et al.* 2008), and submerged soils can sustain an indigenous N supply (INS) for rice as evidenced by long-term stable yields in minus-N plots in long-term experiments. Paddy soils historically cultivated with rice monoculture using puddling and soil submergence can be susceptible to loss of built-up SOM and INS when converted to the production of aerobic rice. On the other hand, SOM in paddy soils historically tilled aerobically for the production of upland crops — such as wheat and maize — might have already stabilized at relatively lower levels than for soils without such aerobic tillage. Conversion to aerobic rice on such soils might consequently have relatively less risk of loss of SOM. Resource-conserving technologies for rice aim to prevent further loss of SOM and potentially slowly build up SOM.

As a general principle, fertilizer N, P, and K requirements for a given target yield could be higher for rice grown on aerobic soil than submerged soil. A higher need for fertilizer N can arise from lower INS due to lower BNF and possible lower net N mineralization in aerobic soil. A higher need for fertilizer P can arise from the reduced availability of soil P in aerobic soil. A higher need for fertilizer K can arise from reduced input of K with irrigation water due to water savings with aerobic rice. Soil aeration increases zinc availability on acid soils, but it can decrease zinc and iron availability on high-pH soils, leading to a need for iron and zinc fertilization for dry-seeded aerobic rice (Malik and Yadav 2008).

### **Switching from continuous rice to diversified cropping systems**

Even with water-saving technologies for rice cultivation, the water requirements for rice remain higher than for other cereal crops. Diminishing supplies of irrigation water and opportunities for income from non-rice crops can serve as drivers for diversification from the production of rice monoculture with soil submergence to a rotation of rice with other crops such as maize grown on well-drained aerobic soils.

Continuous rice with puddling and soil submergence sustains SOM and INS (Pampolino *et al.* 2008), but the conversion from continuous rice to a rice–upland crop rotation with conventional tillage of aerobic soil can lead to a loss of SOM. In a long-term experiment in the Philippines, the conversion from rice–rice to rice–maize led to a loss of SOM, which increased with aerobic soil tillage. Nitrogen balances were also more negative with rice–maize than with rice–rice (Buresh, unpublished). Resource-conserving technologies with reduced or zero tillage for establishment of the upland crop merit further investigation for sustaining SOM and INS built up during the historical cultivation of continuous rice with soil submergence.

Whereas ammonium is the stable form of inorganic N in submerged soils, nitrate accumulates in aerobic soils. The accumulated nitrate is prone to loss by leaching and denitrification with the formation of nitrous oxide when soil, after production of an upland crop, is submerged during land preparation for subsequent rice production (Buresh *et al.* 2008). This potentially greater loss of soil N combined with less input of N by BNF could result in reduced INS when continuous rice is converted to a rice–upland crop rotation.

### **Environmental concerns**

Soil submergence promotes the production of methane by anaerobic decomposition of SOM and added organic materials, whereas aeration of soil reduces methane emissions. Emissions of nitrous oxide, another greenhouse gas with a higher global warming potential than methane, are typically negligible or low during continuous soil submergence. Soil aeration increases the formation of nitrate and emissions of nitrous oxide.

Therefore, water-saving technologies for rice production, such as AWD and aerobic rice, and the inclusion of more upland crops in the cropping system can reduce emissions of one greenhouse gas (methane) while increasing the emissions of another more potent greenhouse gas (nitrous oxide). A greenhouse study suggested that continuous soil submergence has a lower risk of combined methane and nitrous oxide emissions than AWD when crop residues are not incorporated, but AWD has a comparable or lower risk than continuous soil submergence when crop residues are incorporated (Johnson-Beebout *et al.* 2009). Future research should identify combinations of water, residue, and crop management with the lowest global warming potential from combined methane and nitrous oxide emissions.

Weeds and nematodes are typically more serious constraints on aerobic soils than on submerged soils, potentially necessitating increased pesticide use. The accumulation and leaching of nitrate in aerobic soils could cause an increased nitrate contamination of the groundwater.

## Conclusions

Paddy soils in the future will increasingly be managed within environments of diminishing and erratic water supplies. Researchers are striving to develop crop, water, and residue management practices that help grow rice with less water and enable more diversified rice-based cropping systems. The developed management practices must simultaneously ensure the production of sufficient affordable rice and profitability for producers. Researchers will be increasingly challenged to document the effects of new management practices on C storage in soil, nutrient cycling, greenhouse gas emissions, and soil nutrient supplying capacity. When new management practices unavoidably lead to declines in indigenous nutrient supply from levels obtained for continuously submerged soils, existing recommendations for nutrient inputs must be appropriately tailored to meet emerging needs. Researchers should also aim to better understand critical threshold durations and frequencies of soil submergence for additional critical ecosystem services such as control of weeds, nematodes, and other pests.

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# Effect of Nitrogen sources on Aerobic Rice production under various rice soil Eco systems

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## Abstract

A field experiment was conducted at the Wetlands of Tamil Nadu Agricultural University, Coimbatore during *Kharif* season of 2008 with an objective of assessing the effect of different eco systems of rice cultivation and N sources on the growth and yield of CORH-3 hybrid rice. Alternate wetting and drying and flooded rice systems of cultivation were on par with each other in terms of growth characters, yield attributes and grain yield of hybrid rice. Application of 50% of N as Urea + 50% of N as poultry manure recorded higher growth characters, yield attributes and grain yield which was closely followed by application of 100% of N as Urea. The highest water productivity was achieved by adopting alternate wetting and drying system of cultivation. Higher profit was obtained with 50% of N as Urea + 50% of N as poultry manure in an alternate wetting and drying system of rice cultivation followed by application of 100% of N as Urea in the alternate wetting and drying regime.

## Key Words

Nutrient management, alternate wetting and drying, flooded rice ecosystem

India is the first among countries other than China to develop and commercialize hybrid rice technology (Siddiq, 2002). Scarcity of freshwater resources has threatened the production of the flood-irrigated rice crop. By 2025, 15 out of 75 million hectare of Asia's flood-irrigated rice crop will experience water shortage (Tuong and Bouman, 2003). To reduce water use in irrigated rice, water-saving regimes can be introduced, that aim to reduce non-beneficial water flows from rice fields during crop growth namely seepage, percolation and evaporation by Alternate Wetting and Drying (AWD) irrigation and aerobic rice system (Bouman *et al.*, 2005). Nearly 50 per cent gain in food grain productivity seen in recent times has come through adoption of fertilization practices alone. Although the performance of hybrid rice was studied under submerged conditions for agronomic yield, minimal efforts have been made to study its performance in different soil eco systems viz., alternate wetting and drying and aerobic condition. Therefore the present investigation was undertaken to study the effect of different sources of N under various systems of rice cultivation.

## Materials and methods

A field experiment was conducted during 2008-2009 at the Wetland Farm of Tamil Nadu Agricultural University, Coimbatore. The soil was deep clay loam with soil PH, electrical conductivity, organic carbon and soil available N, P and K were 7.3, 0.46%, 0.64% and 244, 17.2 kg ha<sup>-1</sup> and 505 kg ha<sup>-1</sup> respectively. The experiment was laid out in split -plot design replicated thrice. The main plots represented three types of rice cultivation eco systems, viz. 'Aerobic rice', 'Alternate wetting and drying' and 'Flooded rice'; the sub plot consisted of five different nitrogen treatments, viz. 100% of N as Urea, 100% of N as Ammonium sulphate, 50% of N as Urea + 50% of N as poultry manure, 50% of N as Ammonium sulphate + 50% of N as poultry manure and 100% of N as poultry manure. The recommended dose of 175:60:60 kg of NPK was applied to all the three systems of rice cultivation. However, different sources and combinations of manures were used while applying to subplots. A dose of 50% N along with 100% P and 100% K was applied as basal dose in all the subplots except subplot 5 where 100% N as poultry manure was applied as basal dose. Well-decomposed poultry manure was used and applied on a N basis only. The entire dose of 60 kg P<sub>2</sub>O<sub>5</sub> per hectare was applied basally in the form of single super phosphate in all the subplots. Potassium at 60 kg ha<sup>-1</sup> was applied uniformly to all the plots irrespective of the treatments.

## Results and discussion

### Growth parameters

Growth parameters like plant height, tillers, root length, root volume and dry matter production of hybrid rice were comparatively higher under the alternate wetting and drying rice cultivation method. This might be due to high soil aeration. In alternate wetting and drying, the action of micro organisms can be promoted and the

accumulation of poisonous substances in the soil can be avoided by favourable soil aeration (Mao Zhi, 1997). The number of tillers, root length and root volume were found to be the highest with the application of 50% of N as Urea + 50% of N as poultry manure and it was comparable with 100% of N as Urea. It might be due to the improved soil physical properties *viz.*, bulk density, infiltration rate, hydraulic conductivity by organic manures thereby the soil health favoured better growth attributes.

### **Yield attributes**

Productive tillers, panicle length and number of filled grains were higher under alternate wetting and drying system of rice cultivation due to better aeration and microbial activity. Soil drying during grain filling and also enhanced mineralization of soil organic matter and thus N release, which is good in the short term but reduces soil fertility in the long term.

Enhances the partitioning of assimilates from vegetative tissues to grains. This is in line with the findings of Hao Zhang *et al.* (2008). Higher yield attributes were registered with 50% of N as Urea + 50% of N as poultry manure and comparable with 100% of N as Urea. More number of early formed tillers under steady supply of N due to mineralization of poultry manure with 50% of N as Urea increased the productive tillers and thereby increased the numbers of panicles. This is mainly because of organic manures have low nutrient content and slow release of N and Poultry manure usually contains a lot of P so that in these treatment much more P was applied which explains the observed results.

An appropriate combination of organic and inorganic nutrient sources was found to enhance the efficiency of nutrients and ultimately increased the growth and yield attributes of rice as reported by studies of earlier workers. (Maragatham, 1996; Battacharya and Nain, 2001).

### **Yield**

A higher grain yield was obtained with the alternate wetting and drying system of cultivation and it was on par with flooded rice (Table 1). This was due to the increased value of yield attributing characters like panicle number, panicle length, thousand grain weight and low sterility percentage. A similar finding was reported by Viraktamath (2006). Rice grain yield obtained from 50% of N as Urea + 50% of N as poultry manure was found to be the highest and it was on par with 100% of N as Urea. This might be due to continuous and steady supply of N into the soil solution to meet the required nutrients for physiological processes, which in turn improved the yield. Also increased nutrient uptake especially of N and P resulted in increased photosynthetic rate and increased plant growth. Increased photosynthetic rate resulted in higher translocation to sink and more grain yield.

### **Nutrient uptake**

The alternate wetting and drying system recorded more nutrient uptake due to enhanced root activity as evident from the presence of longer roots and higher root volume which in turn increased total dry matter production and nutrient uptake (Rajesh and Thanunathan, 2003). Improved uptake of NPK was observed with 50% of N as Urea + 50% of N as poultry manure. The N mineralized during the decomposition of organic manures would have enhanced N availability in the rhizosphere resulting in increased nutrient uptake by rice and resulting in increased dry matter.

### **Water productivity**

Water productivity is the term used to express the incremental crop yield and income for every unit volume of water used. The alternate wetting and drying system of rice cultivation resulted in the highest water productivity ( $5.66 \text{ kg ha}^{-1} \text{ mm}^{-1}$ ) and the lowest water productivity was recorded for the flooded rice method (Table 2). The alternate wetting and drying system of rice cultivation resulted in higher yield for the moderate quantity of water consumed. This is in line with the findings of Tran Thi Ngoc Huan *et al.* (2008).

**Table 1. Effect of N sources and various eco-systems of cultivation on grain yield, straw yield (kg ha<sup>-1</sup>) and harvest index of hybrid rice.**

Treatments	Grain yield (kg ha <sup>-1</sup> )	Straw yield (kg ha <sup>-1</sup> )	Harvest index
<b>Systems of rice cultivation</b>			
M <sub>1</sub>	3974	6148	0.35
M <sub>2</sub>	6949	10841	0.37
M <sub>3</sub>	6206	10412	0.35
SEd	295.5	525.9	0.004
CD (P=0.05)	797.8	1085.5	0.010
<b>N sources</b>			
S <sub>1</sub>	5793	10623	0.36
S <sub>2</sub>	5415	8934	0.34
S <sub>3</sub>	6020	10991	0.38
S <sub>4</sub>	5610	9339	0.33
S <sub>5</sub>	5193	8069	0.33
SEd	270.6	519.2	0.01
CD (P=0.05)	558.4	1069.6	0.02
<b>Interaction</b>			
M X S      SEd	422.4	844.3	0.02
CD	NS	NS	NS
S X M      SEd	468.6	910.9	0.02
CD	NS	NS	NS

Main plot	Sub plot
M <sub>1</sub> - Aerobic rice M <sub>2</sub> - Alternate Wetting and Drying M <sub>3</sub> - Flooded rice	S <sub>1</sub> - 100% of N as Urea S <sub>2</sub> - 100% of N as Ammonium sulphate S <sub>3</sub> - 50% of N as Urea + 50% of N as poultry manure S <sub>4</sub> - 50% of N as Ammonium sulphate + 50% of N as poultry manure S <sub>5</sub> - 100% of N as Poultry manure

**Table 2. Water productivity (kg ha<sup>-1</sup> mm<sup>-1</sup>) of hybrid rice under different eco systems of cultivation.**

Treatments	Total water used ( mm )	Water productivity (kg ha <sup>-1</sup> mm <sup>-1</sup> )
M <sub>1</sub>	764	5.20
M <sub>2</sub>	1227	5.66
M <sub>3</sub>	1543	4.02
Mean	1178	4.96

Data not statistically analysed

M<sub>1</sub> - Aerobic rice  
M<sub>2</sub> - Alternate Wetting and Drying  
M<sub>3</sub> - Flooded rice

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# Effect of wetting and drying on structural regeneration of puddled soil

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## Abstract

The effect of repeated wetting and drying and the degrees of drying on structure regeneration of puddled soil were studied on two soils, e.g., a grey clay and a sandy loam soil. Degrees of drying were arranged by sun drying at different periods, e.g., 2-4 days, 5-8 days and 9-12 days drying, which were corresponding to pF 2.1-2.5, 3.1-3.8 and 4.6-6.0 respectively. Five wetting/drying cycles were conducted during this experiment. Measurements on the effect of different degrees of puddling showed that the two soils responded quite similar towards partially drying. An increased in the degree of drying resulted in faster improvement of soil structure.. Drying the puddled soil to its air dry water content is very effective in the process of structural regeneration. But this study also showed that the regeneration of puddled soil is possible by partial drying.

## Key Words

Puddled soil, wetting/drying, dispersion, structural regeneration

## Introduction

During the rice growing season, wetting and drying cycles influence the reaggregation of soils (Bakti *et al.* 1998) which is beneficial for growing upland crops after rice harvest. Greenland (1981) reported that in wetland rice, continuous flooding and rice cropping cause a breakdown of water stable aggregates resulting in the deflocculation of the soil. When puddled soils are drained soil aggregates reform. This is beneficial for the improvement of aeration and the preservation of soil structure (van de Goor 1974). In studying the aggregation of allophane soils, Kubota (1972) found that drying after flooding resulted in aggregation of silt and clay particles, and that drying was the main factor responsible in these soils. Whereas, White (1966) found that cracks in some soils, which develop during drying, will produce the initial faces of soil aggregates and when soil drying is rapid it is not uniform, leading to unequal stresses and strains resulting in aggregate formation. According to van de Graaff (1978), during rapid drying the stresses developed produce smaller ped than those developed during slow drying, leading to smaller aggregates. Sanchez (1973) suggested that puddled soil must be dried slowly to increase structural aggregation. The objective of this study was to investigate the effect of degree of drying or partial drying on structure regeneration of puddled soil.

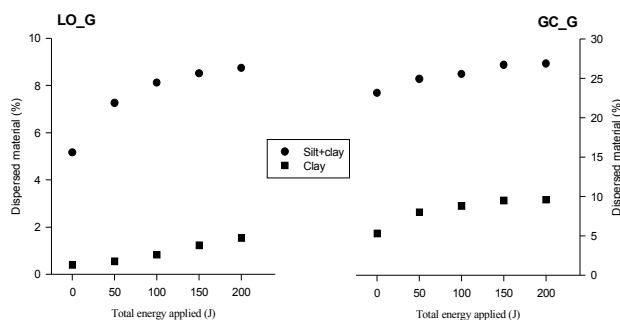
## Materials and methods

Two contrasting soils were used in this experiment: a grey clay from Griffith, NSW (GC\_G) and a sandy loam from the Lockyer Valley near Brisbane, Queensland (LO\_G). The effect of puddling intensity was examined by using air-dried equivalent of 1600 g oven dried soil (<2mm) which was puddled at total energies applied of 0, 50, 100, 150 and 200 Joules. The amount of dispersible silt+clay (<20 µm) and clay (<2 µm) were determined by sedimentation using the pipette method. Saturated hydraulic conductivity ( $K_{sat}$ ) of the puddled soil was measured using a falling head method. After the saturated hydraulic conductivity measurements the soil was drained and dried for 12-14 days of sun drying. Once the soil dried to approximately their air dry water content, the soil was carefully flooded again. The soil was stored in a constant temperature of 20 °C for 48 hr to allow

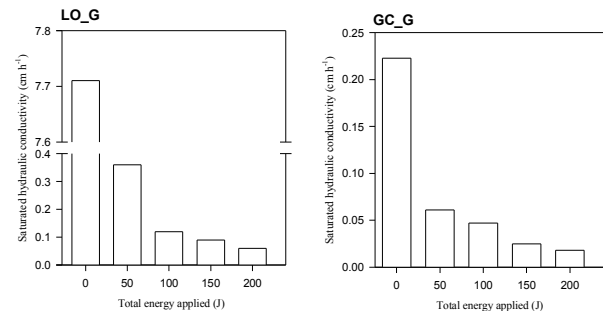
equilibrium before saturated hydraulic conductivity measurement was taken. Samples for the amount of dispersible clay and silt+clay determination were taken from a spare cylinder. After the saturated hydraulic conductivity measurement, the soil was drained again, and 5 wetting/drying cycles was conducted in this experiment. The effect of degrees of drying were investigated where the soil was arranged by sun drying at different periods, e.g., 2-4 days, 5-8 days and 9-12 days drying, which corresponded to pF 2.1-2.5, 3.1-3.8 and 4.6-6.0 respectively. These soil were subjected to a single level of puddling with an applied energy of 50 Joules. Five wetting/drying cycles were conducted for this experiment.

## Results

Puddling of soil results in disaggregation and dispersion of soil materials. Results showed that the amount of dispersed clay or silt+clay increased with increasing puddling energy applied ( $P < 0.05$ ) (Figure 1). Figure 2 showed that there was a significant reduction ( $P < 0.05$ ) in the saturated hydraulic conductivity,  $K_{sat}$ , after

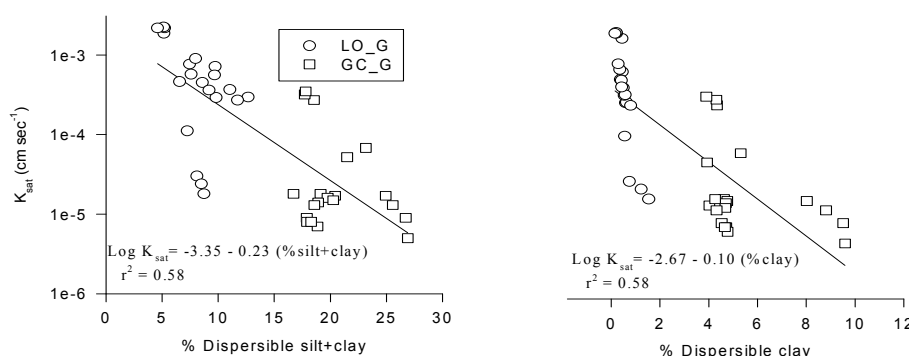


**Figure 1. Effect of puddling on soil dispersion for LO\_G and GC\_G soil.**



**Figure 2. Effect of puddling on saturated hydraulic conductivity ( $K_{sat}$ ) for LO\_G and GC\_G soil.**

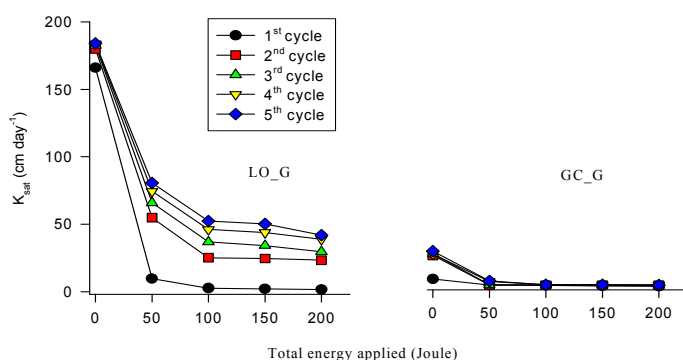
puddling.  $K_{sat}$  decreased rapidly when the soil was puddled with only 50 - 100 Joule energy input. When the soil is puddled with greater energy (100 to 200 Joule) however, the additional reduction in  $K_{sat}$  was small. A large reduction in  $K_{sat}$  of the puddled soil was due to the disaggregation induced by puddling. The greater the soil dispersion, the lower is the saturated hydraulic conductivity (Figure 3). Wetting/drying cycles are known to improve soil structure. Results clearly showed reduction in the amount of silt+clay ( $< 20 \mu m$ ) and clay ( $< 2 \mu m$ ) dispersed during wetting/drying cycles in clay (GC\_G) and sandy loam (LO\_G) soil respectively. Figure 5 and 6 shows the silt + clay data for the two soils.



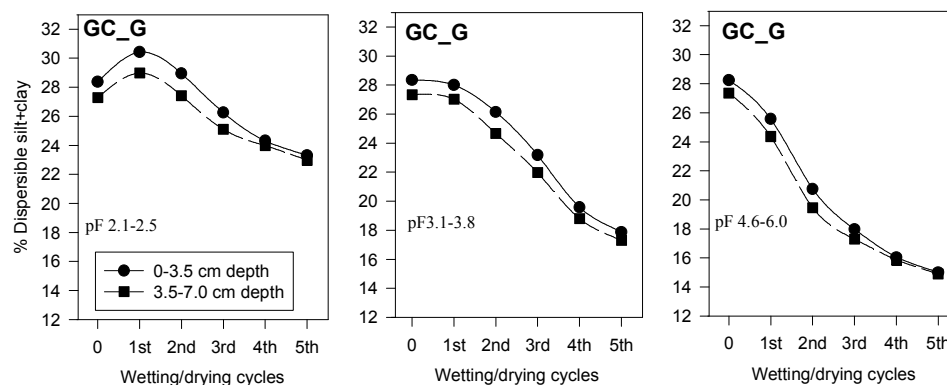
**Figure 3. The relationship between puddled soil hydraulic conductivity and the amount of dispersed silt+clay and clay for LO\_G and GC\_G soil.**

Repeated wetting/drying cycles to air dry water contents increased  $K_{sat}$  of the puddled soil significantly in the fine textured soil but had little effect on the sandy soil (Figure 4). However, in fine textured soil (GC\_G) the

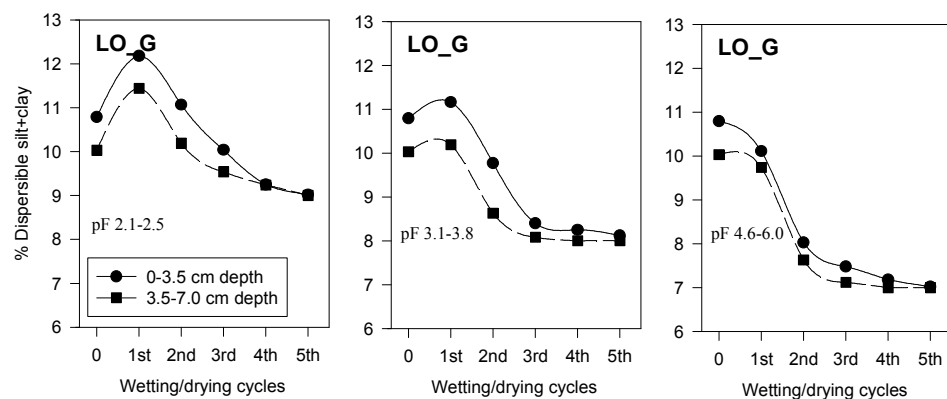
recovery of  $K_{sat}$  was not significant except for the soil puddled with energies less than 100 Joules. This improvement was partly the consequences of rapid wetting. Rapid wetting causes partial slaking by inducing micro-cracks and these micro-cracks has the effect of making the soil easily crumbled. The Combined effect of wetting-drying and degree of drying on the % dispersed silt+clay is shown in Figure 5 and 6 for the surface 3.5 cm of soil. It is clear that the degree of drying has a strong effect on the structural regeneration shown by the decrease in dispersed silt +clay. The greater the drying and the greater the frequency of drying,, the greater is the reduction is dispersed material. In the two soil the amount of  $<20\ \mu m$  and  $<2\ \mu m$  dispersed soil was significantly reduced until the fifth cycle.



**Figure 4. Changes in  $K_{sat}$  during wetting/drying cycles for LO\_G and GC\_G soil.**



**Figure 5. Effect of different degrees of drying on the amount of silt+clay ( $<20\ \mu m$ ) dispersed at 0-3.5 cm and 3.5-7.0 cm depth in GC\_G soil.**



**Figure 6. Effect of different degrees of drying on the amount of silt+clay ( $<20\ \mu m$ ) dispersed at 0-3.5 cm and 3.5-7.0 cm depth in LO\_G soil.**

## Conclusion

Results of this study shows that soil puddling increased dispersion but dispersion decreased with wetting/drying cycles resulting in increased saturated hydraulic conductivity,  $K_{sat}$ , of the puddled soil. However, the improvement in  $K_{sat}$  for fine textured soil was small when intense puddling was applied (the soil was excessively puddle). This suggests that in clay soil where percolation rates are low, puddling, which is capital intensive and detrimental to soil structure, should be minimised. Drying the puddled soil to its air dry water content is very effective in the process of structural regeneration. This study also showed that partial drying will result in lower levels of structural regeneration of puddled soil and may possibly require greater frequency of drying to fully regenerate the soil.

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# Effects on microbial communities of long-term application of organic matter and conversion to upland condition of paddy field: Estimation by eDNA analysis

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## Abstract

Microorganisms and their activities in paddy field soil play important roles for rice production and soil fertility. Effects of long-term application of organic matter and conversion to upland on microbial community structures were investigated in Japanese paddy fields by molecular ecological techniques (eDNA analysis) for better understanding of their roles in paddy field soil. The effect of long-term application of organic matter (rice straw compost) on denaturing gradient gel electrophoresis (DGGE) patterns of 16S rRNA genes of bacterial communities and 18S rRNA genes of fungal communities was small. DGGE banding patterns from 18S rRNA genes of fungal communities in paddy-upland rotational plots and upland plots converted from paddy fields were different from those for plots continuously cropped with paddy rice. These results indicated that the microbial communities in paddy fields were not influenced by long-term application of organic matter, but were affected by conversion to upland fields from paddy fields.

## Key Words

Community, eDNA, fungi, bacteria, paddy field, upland

## Introduction

Rice cultivation in flooded paddy fields is a good and important system of crop production in the monsoon area of Asian countries. Many kinds of physical, chemical and biological functions of soil contribute to growth of rice plants in paddy fields. Soil microorganisms and their activities play important roles in those functions, especially various metabolic reactions in soil such as mineralization of soil organic nitrogen and decomposition of rice straw and compost applied to soil, which support rice production as well as maintain the fertility of paddy soil. Therefore, it is important to elucidate microbial community structures in paddy fields in order to understand the roles of microorganisms.

In the previous studies, we showed the stability of bacterial communities in paddy field soil throughout a year including the cultivation periods of rice under flooded conditions and of wheat under upland conditions by the denaturing gradient gel electrophoresis (DGGE) analysis based on DNA and RNA (Kikuchi *et al.*, 2007). In this communication, we present effects of long-term application of organic matter (rice straw compost) and conversion to upland on microbial communities in Japanese paddy fields revealed by molecular ecological studies (eDNA analysis).

## Materials and Methods

### *Study site*

Three experimental fields with long-term application of organic matter (for 32-44 years), Aomori Prefectural Agriculture and Forestry Research Center in Kuroishi, Aomori, Japan (latitude 40°38'N, longitude 140°34'E; Anthraquic Kuroboku soils; Melanaquand) (Kuroishi), National Agricultural Research Center for Tohoku Region in Daisen, Akita, Japan (latitude 39°29'N, longitude 140°29'E; Gray Lowland soils; Typic Fluvaquents) (Omagari) and National Agricultural Research Center for Kyushu Okinawa Region in Chikugo, Fukuoka, Japan (latitude 33°12'N, longitude 130°29'E; Gray Lowland soils; Endoquepts) (Chikugo), were used and soil samples were taken from the plots applied with chemical fertilizer (CF) and with rice straw compost plus chemical fertilizer (RSC) for the analyses of soil bacterial and fungal communities in 2006 and 2008. The fields were continuously cropped with only rice in Kuroishi and Omagari or under double cropping with rice and wheat in Chikugo. The samples collected from paddy-upland rotational fields in the

National Agricultural Research Center for Tohoku Region and upland fields converted from paddy fields in the National Agricultural Research Center for Kyushu Okinawa Region in 2008 and 2009 (Table 1) were also subjected to the analysis of soil fungal communities.

**Table 1. Cropping system of fields under paddy-upland rotation in Omagari and conversion to upland in Chikugo.**

	Plot	1968-1981				1982-1999				2000-2002				2003-2004				2005-2007				2008-2009			
Omagari	Paddy-upland rotation	R				S				R				S				R				S			
	Field A	Cont. paddy				R				R				R				R				R			
		-89	90	91 92	93	94	95	96	97 98	99	00	01 02	03	04	05 06	07	08	09							
Omagari	Rot.1	R	S	R	R	S	R	S	R	S	R	S	R	R	S	R	S	R							
	Field B	Rot. 2	R	S	S	R	S	S	S	R	S	S	S	R	S	S	R	S	S						
		Cont. paddy	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R						
Chikugo		Plot				-2004				2005 - 2009															
		Upland converted				R				S (-W) (Double cropping)															
		Cont. paddy				R				R															
R, Paddy rice ; S, Soybean ; W, Wheat																									

**R, Paddy rice ;S, Soybean ;W, Wheat**

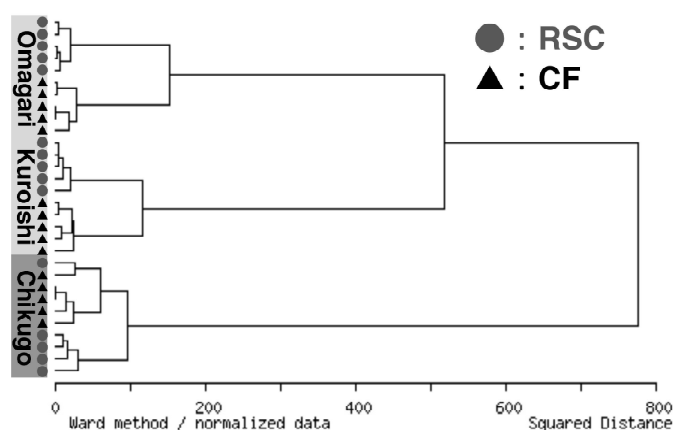
### DGGE analysis

Soil bacterial and fungal communities were analyzed by DGGE of 16S rRNA and 18S rRNA gene fragments, respectively. Variable regions (V3 and V6-V8 for bacteria and V1-V2 for fungi) of 16S rRNA and 18S rRNA gene fragments were amplified with PCR from DNA extracted from the soils and subjected to the DGGE (Muyzer *et al.*, 1993; 1998; Hiramatsu *et al.*, 2007; Morimoto and Hoshino, 2008). Bacterial and fungal communities were compared between the plots based on the analysis of the DGGE banding patterns by cluster analysis and principal component analysis.

## Results

### *Effect of long-term application of rice straw compost on microbial communities in paddy field soils*

The DGGE banding patterns of 16S rRNA gene fragments in the V3 and V6-V8 regions retrieved from bacterial communities were not greatly different between CF and RSC plots of the experimental fields with long-term application of organic matter in Kuroishi, Omagari and Chikugo. The patterns did not show conspicuous changes throughout the rice growing periods in the respective fields. Figure 1 shows the cluster analysis of the banding patterns of 16S rRNA gene fragments in the V6-V8 regions. Although some differences were found in the patterns among the three locations, the differences were mainly attributed to variations in the band intensities and most of the DGGE bands were commonly present through the samples obtained from the three locations.

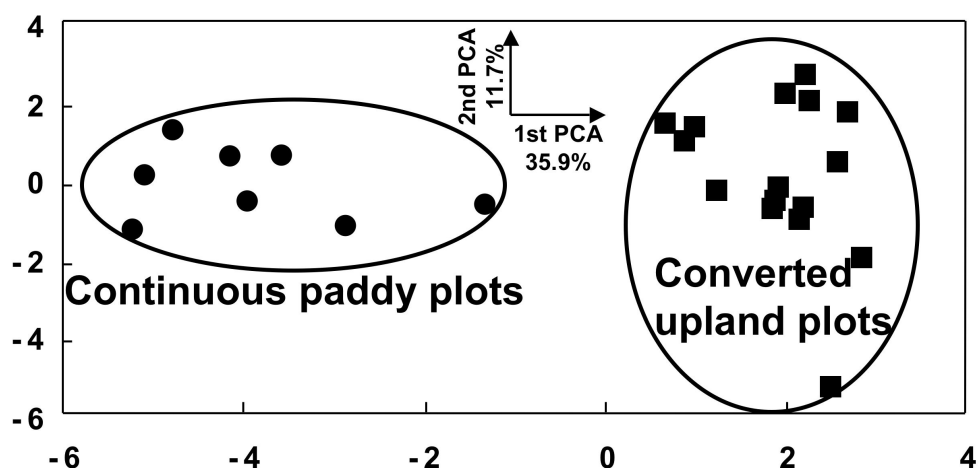


**Figure 1.** Cluster analysis of DGGE pattern of the 16S rRNA gene fragments in the V6-V8 regions retrieved from bacterial communities in the experimental fields (Kuroishi, Omagari and Chikugo) with long-term application of organic matter in 2006 (Hiramatsu *et al.*, 2007). CF, chemical fertilizer plot; RSC, rice straw compost plot.

The DGGE banding patterns of 18S rRNA gene fragments retrieved from fungal communities were also similar between CF and RSC plots of the experimental fields with long-term application of organic matter in Kuroishi, Omagari and Chikugo in 2006 and 2008 (data not shown). The patterns did not show conspicuous changes throughout the rice growing periods in the respective fields. These results show that bacterial and fungal communities in paddy field soil were not influenced by long-term application of rice straw compost and field managements during rice growing periods.

#### *Effect of conversion to upland of paddy fields on fungal communities*

The DGGE banding patterns of 18S rRNA gene fragments retrieved from fungal communities were different between upland plots converted from paddy fields in Chikugo or paddy-upland rotational plots in Omagari and the plots continuously cropped with paddy rice. Figure 2 shows the principal component analysis of the banding patterns of 18S rRNA gene fragments in Chikugo fields. These results show that fungal communities in paddy field soil were influenced by conversion to upland from paddy fields and were different from the communities in paddy fields.



**Figure 2.** Principal component analysis of DGGE pattern of the 18S rRNA gene fragments retrieved from fungal communities in upland plots converted from paddy field and paddy plots continuously cropped with paddy rice in Chikugo in 2008 and 2009.

#### **Conclusion**

Composition of bacterial and fungal communities in paddy fields was not influenced by long-term application of organic matter, but was affected by conversion to upland fields from paddy fields. The stable characteristics of soil microbial communities may be attributed to rice cultivation under flooded conditions in paddy fields and may play some important roles in productivity and sustainability of rice cultivation in paddy fields. Thus, it is interesting to elucidate the mechanisms that maintain the stability of microbial communities in paddy field soil.

## Acknowledgement

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# Increase sorption endosulfan by soil amendments and its effects on retention and leaching from soil

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## Abstract

Sorption of pesticides to substrates used in biopurification systems is an important control on the system's efficiency. Ideally, pesticide sorption should occur fast so that leaching of the pesticide in the biopurification system is minimized. This study investigated the sorption and leaching of endosulfan on substrates commonly used in a biopurification system, i.e. manure, sugar cane compost and clay loam soil. The distribution of endosulfan along the soil profile, obtained from soil column experiments, indicated that the amount endosulfan retained ranged from 68.4% for the column filled with the original soil to 92.4% for that filled with the organic amended soil. Amounts of endosulfan recovered in the leachates, which ranged from 7.7% (organic amended soil) to 23.7% (unamended soil) of that applied, depended upon the loading rate and the source of organic amendment. Organic amendments significantly reduced the leaching of endosulfan and compost amended soil showed a higher potential than manure. It can be concluded that organic amendment may be an effective management practice for controlling pesticide movement. In fact, organic matter with strongly adsorbing sites can prevent endosulfan movement.

## Key Words

Biopurification, Endosulfan, soil amendments, sorption, leaching.

## Introduction

Endosulfan is a chlorinated pesticide of the cyclodiene group. It is being used extensively throughout the world for the control of numerous insects in a variety of food and non-food crops. It is extremely toxic to fish and aquatic invertebrates, and is a priority pollutant for international environmental agencies. It has also been detected in the atmosphere, soils, sediments, estuaries, surface and rain waters, and food stuffs. As endosulfan is found in ground waters, it is apparent that there is significant mobility of these chemicals through the soil (Kumar *et al.* 2006). The displacement of pesticides from soil to water strongly depends on the extent to which they are retained in soils, which in turn depends on the adsorption and desorption properties of the soil (Si *et al.* 2006). A possible approach to reduce the direct contamination of water bodies by pesticides using on-farm biopurification systems. The matrix in a biopurification system is composed of a material with a good porosity retaining water and air (e.g. soil, peat, green waste compost), an easily degradable organic material as the source of nutrients. Knowledge of the pesticide adsorption characteristics of soil is necessary for predicting their mobility and fate in soil environments and also to understand whether bioremediation is a feasible option for the clean up of contaminated soil. It has not been studied in detail for endosulfan which is the most commonly used pesticide in Iran. The objectives of the study were 1- Adsorption of endosulfan by soil with sugar cane compost and manure 2-Leaching of endosulfan by soil with Sugarcane compost and manure.

## Methods

The soil chosen for this study was collected from the surface of Ahvaz agricultural college field, air-dried, and passed through 2-mm sieve. The composition of the soil was: sand 22.5%, silt 38.3%, clay 39.2%, and organic matter 0.63%, cation exchange capacity (CEC) 17.39 cmol kg<sup>-1</sup>, pH (1:1, H<sub>2</sub>O) 7.41. Soil columns treatments were prepared by adding organic amendments in 2 levels of 0, 50 T/h equivalent to field application. The organic amendments manure and sugar cane compost were used. The samples of the amended soil so obtained were analyzed for organic carbon (OC), pH, and cation exchange capacity (CEC) and some chemico-physical soil properties. Irrigation was performance according two pore volume of distil water; the leachate from each column was collected for 8 weeks. The concentrated extract was analyzed for endosulfan by GC-ECD. The adsorption experiments were carried out as follows: endosulfan solutions containing initial pesticide concentration (C<sub>0</sub>) between 2 mg/L and 4 mg/L were prepared. Spiked soil samples were prepared by adding 25 mL of each endosulfan solutions to 10 g of each soil sample. Then, 10 ml of acetone was added and suspension was mixed for 30 min with a mechanical shaker. After the bulk of

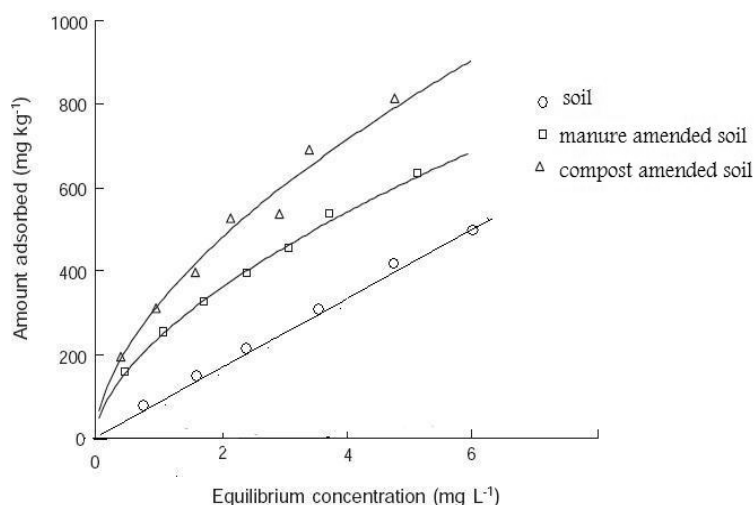
the solvent was evaporated at room temperature, the sample was stored at 4 °C in stopper conical flask for 3 day. Then, the extractions were carried out and the amount of extracted endosulfan was determined by GC-ECD.

## Results

Adsorption isotherms of endosulfan on soil and manure and sugarcane compost amended soil are shown in Figure 1. The adsorption data are fitted to the Freundlich equation:  $C_s = K_f C_e^n$ . Where  $C_s$  (mg/kg) is the amount of endosulfan adsorbed,  $C_e$  (mg/L) is the equilibrium concentration in solution, and  $K_f$  and  $n$  are empirical constants which are presented in Table 1. Adsorption capacity ( $K_f$ ) values range from 4.36 for the original soil to 5.74 for compost amended soils. In Table 1, the  $K_d$  values which have been calculated from the fit of the experimental sorption isotherms ( $C_s = K_d C_e$ ) are presented. The  $K_{OC}$  constants are calculated for the herbicide and the soils studied [ $K_{OC} = (K_d / OC\%) \times 100$ ].  $K_{OC}$  values of sugarcane compost is higher than of manure amended soil (Table 1). Similar results were obtained by Barriuso *et al.* (1997) for eight pesticides in freshly amended soil, and by Sluszný *et al.* (1999), for three pesticides in freshly amended and incubated soils.

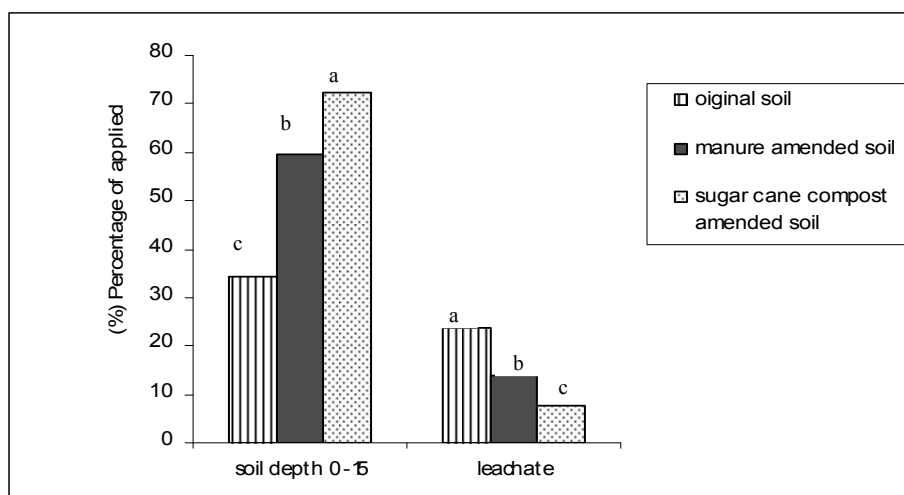
**Table 1-Parameters of the Freundlich equation,  $K_d$ , and  $K_{OC}$  values for endosulfan in soil, manure, sugarcane compost**

Sorbent	OC%	$K_f$	$n$	$r$	$K_d$	$r$	$K_{OC}$
Soil control	0.63	4.36	0.76	0.971	1.98	0.935	314
Sugarcane compost	1.22	5.23	0.72	0.991	2.42	0.992	198
Manure	1.38	5.74	0.73	0.992	2.44	0.983	180



**Figure 1. Adsorption isotherm of endosulfan on soil, manure and sugar cane compost amended soil.**

The amount of endosulfan retained range from 34.5% for the original soil to 72.4% for sugarcane compost amended soil (Figure 2). Recoveries of endosulfan in leachate range from 23.7% from the soil without amendment to 7.7% from the soil amended with sugarcane compost soil. The amount of endosulfan in leachate decreases with the increase sugarcane compost addition to soil. Similar results are obtained from the soil amended with manure (Figure 2). For organic amended treatments, most of the residual endosulfan is retained in the upper soil column after leaching. In Figure 2, it can also be seen that the highest recovery efficiencies of endosulfan are obtained in the first 15 cm of the column for sugar cane amended soil and 15 cm of column for manure amended soils. This result may be attributed to stronger adsorption by sugarcane compost than by manure.



**Figure 2. Distribution of endosulfan in the columns filled with original soil, sugar cane compost amended soil and manure amended soil and in leachate.**

## Conclusion

The results showed that the effects of sugar cane compost and manure addition to soil increase endosulfan adsorption, due to the high adsorption capacity of the added organic matter. Organic amendments effectively reduced endosulfan movement in the soil. The reduction in leaching is achieved through increases in sorption. Manipulation of sorption potential in the soils relatively poor in organic matter by amendment with C-rich waste may be an effective management practice for controlling pesticide movement.

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# Irrigation water productivity of rice grown with resource conservation technologies

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## Abstract

Rice being one of the biggest users of world's developed fresh water resources, the agricultural scientists are facing a big challenge to improve its irrigation water productivity ( $WP_{IW}$ ) so as to arrest the declining surface and ground water resources. Different resource conservation technologies (RCTs) are being developed and evaluated for their suitability both in submerged and aerobic system of rice production. This paper highlights the irrigation water use when practising the resource conservation technologies under different irrigation scenarios. The intermittent irrigation scheduling on the basis of soil matric tension (16 kPa at 20 cm depth) could save irrigation water by 30%. Growing of puddle transplanted rice (PTR) on raised beds does not help save irrigation water when similar irrigation schedules are followed in both in direct seeded rice (DSR) and PTR with full furrow-depth of irrigation water. Applying water with half furrow depth could help in improving irrigation water productivity. The water balance for direct-seeded rice needs to be computed under differential irrigation scenarios to achieve highest irrigation water productivity.

## Key Words

Dry-direct seeded rice, intermittent irrigation, raised beds, soil matric potential.

## Introduction

Changing global climatic patterns coupled with declining surface and ground water resources (Hira 2009) have put agriculture on the back foot. The most densely populated Asian countries consume and grow staple rice mainly under submerged conditions (Kukal and Aggarwal 2003) leading to its lower irrigation water productivity ( $WP_{IW}$ ) (Humphreys *et al.* 2007). Since rice is one of the biggest users of world's developed fresh water resources (Tuong and Bouman 2003), the agricultural scientists are facing a big challenge to improve its  $WP_{IW}$  so as to check the decline of surface and ground water resources. Different resource conservation technologies (RCTs) are being developed and evaluated for their suitability both in submerged and aerobic system of rice production (Kukal *et al.* 2005; Humphreys *et al.* 2008). However, whether these RCTs really help save irrigation water is a point of debate, particularly when comparisons are made between similar irrigation schedules in rice grown with RCTs and conventional systems. For example rice grown under unpuddled conditions may consume higher irrigation water, if irrigated with the similar schedule as in puddled system. Thus irrigation needs to be rescheduled for different RCTs particularly when shifting from anaerobic system to aerobic system.

This paper aims to highlight the irrigation water dynamics in rice grown with different water conservation techniques viz. optimum transplanting time, intermittent irrigation at fixed day interval and more precisely at an optimum soil matric potential (SMP), furrow system of irrigation with rice grown on raised beds (fresh and permanent) and direct dry-seeded rice in medium textured soils.

## Materials and methods

### *Soil matric potential based irrigation*

The scheduling of irrigation intermittently at an interval of 2-d was compared with soil matric tension based irrigation (16 kPa at 20 cm depth) in rice raised as described above. These techniques were compared with the farmers' practice of continuous flooding at least for first 50-60 days. The soil matric potential was measured with locally fabricated vacuum gauge tensiometer.

### *Furrow irrigation in raised beds*

Field experiments were carried out in Punjab, India, during 2002-2006 to compare irrigation water use and productivity of transplanted rice and drill-sown wheat on fresh and permanent beds and conventionally tilled flats. The experiments were conducted on deep alluvial sandy loam and loam (Ustochrept) soils in small replicated plots and in large farmer field blocks. The same irrigation scheduling rules were applied to beds and flats, for respective crops. In rice, this involved daily irrigation for the first two weeks after transplanting, followed by irrigation 2 d after the floodwater had dissipated from the surface of the flat plots or furrows. The rice crop was transplanted on 30 cm wide raised beds (both permanent and fresh beds) with furrow (37.5 cm) in between, prepared with a bed planter. The 30-d old seedlings were transplanted in the centre of the slope of the raised beds. The fresh beds were prepared afresh every time after knocking down the previous beds, whereas the permanent beds were not knocked down. These were reshaped before the sowing/ transplanting of the crops. The rice grown on raised beds was compared with the conventional puddled transplanted rice on flats for its irrigation water productivity with different irrigation schedules.

## Results

### *Soil matric potential based irrigation*

The most common method of irrigation in northwestern India is through alternate wetting and drying with a fixed irrigation interval, irrespective of soil type and climatic demand resulting in over-irrigation or under-irrigation under different soil and weather situations. Soil matric potential may be an ideal criterion for irrigation, since variable atmospheric vapour pressure deficit, soil texture, cultural practices and water management affect rice irrigation water requirements. The grain yield of rice remained unaffected up to soil moisture suction of  $16 \pm 2$  kPa each year (Table 1). Increasing soil matric suction to  $20 \pm 2$  and  $24 \pm 2$  kPa decreased rice grain yield non-significantly by 0–7% and 2–15%, respectively, over different years compared to the recommended practice of the 2-day interval for scheduling irrigation. Irrigation at  $16 \pm 2$  kPa soil matric suction helped save 30–35% irrigation water compared to that used with the 2-day interval irrigation.

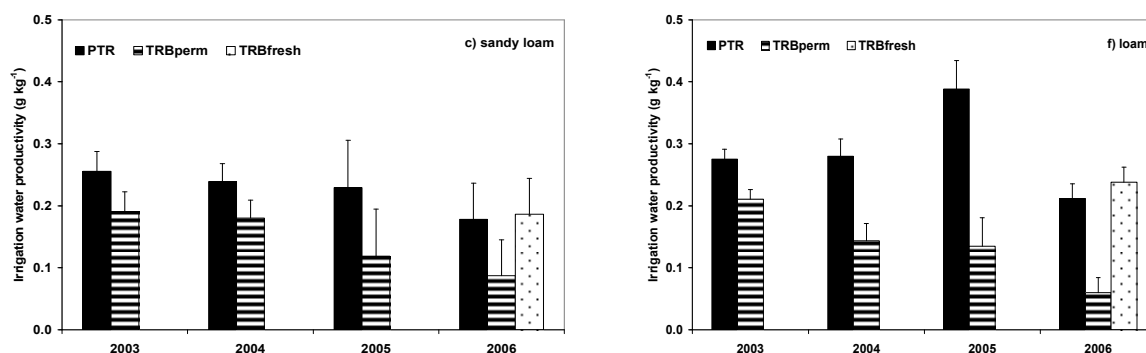
**Table 1. Performance of paddy crop and consumption of irrigation water in relation to soil matric tension based irrigation scheduling**

Soil moisture tension (kPa)	Paddy yield (t/ha)	Irrigation water use (cm)	Irrigation water saving (%)
$10 \pm 2$	6.44	111.8	24.6
$16 \pm 2$	6.40	102.3	31.0
$20 \pm 2$	6.21	89.5	39.6
$24 \pm 2$	5.61	80.0	46.1
2-d fixed interval	6.43	148.3	--

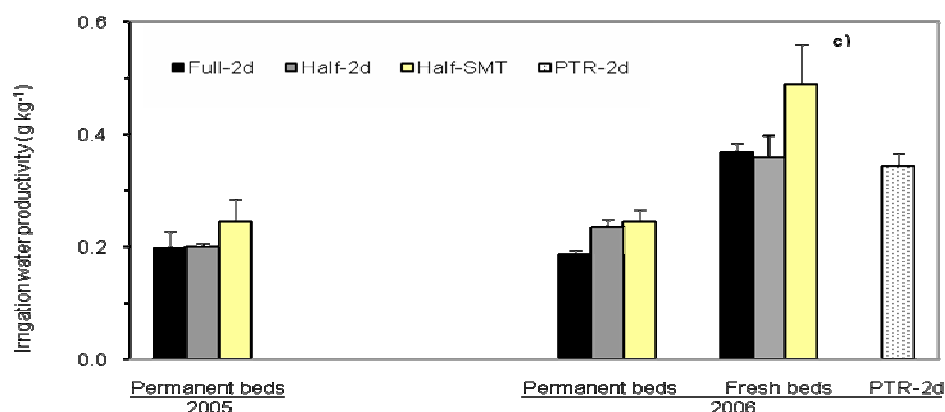
### *Furrow irrigation in raised beds*

Raised beds have been proposed for rice-wheat (RW) cropping systems in the Indo-Gangetic Plains as a means of increasing irrigation water productivity, among many other potential benefits. The amount of irrigation water applied to rice on permanent beds and puddled transplanted rice (PTR) was similar in the small plots on the sandy loam. However, on the loam, irrigation applications to the permanent beds were

always higher than the puddled plots, by 16 to 21%. Over 4 years,  $WP_{IW}$  of transplanted rice on permanent beds decreased with time on both soils, mainly due to declining grain yield as the beds aged (Figure 1). Irrigation applications to fresh beds were lower than to the puddled flats (by 11% on the sandy loam, and 20-24% on the loam) while yields were only 7 and 15% lower, resulting in similar  $WP_{IW}$  on fresh beds and PTR. Reducing irrigation application from full-furrow to half-furrow depth in the farmers' field reduced the irrigation amount on both permanent and fresh beds by 40-50%, but also reduced yield by about 20% (Figure 2).



**Figure 1. Irrigation water productivity of rice on permanent and fresh raised beds in small replicated experimental plots of (a) sandy loam and (b) loam**



**Figure 2. Irrigation water productivity of rice on permanent and fresh raised beds in farmers' scale field plots in loam**

## Conclusion

The studies indicate that the rice crop to be grown with resource conservation technologies can help save irrigation water provided the irrigation scheduling is re-worked for these techniques. Secondly the intermittent irrigation at a fixed time interval is not a feasible practice particularly during present day situation when the climate is becoming so uncertain. Thus the use of soil matric potential as a tool to schedule irrigation is a best bet in saving irrigation water as it indicates most precisely the irrigation time. Thus for each resource conservation technology to grow rice crop, the water balance parameters need to be worked out under different soil and climatic scenarios as a pre-requisite to decide upon the most efficient irrigation scheduling.

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# Microbial biomass and organic carbon stock in paddy soils in the lake Biwa basin, Japan

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## Abstract

The Shiga prefectural government has been promoting environmentally conscious agriculture through a direct payment system in order to conserve water quality of the Lake Biwa, the largest lake in Japan. Furthermore, we target to halve the emission of greenhouse gases by 2030 from the 1990 level. Therefore we have participated in the National Soil Survey Program for monitoring soil carbon content, and investigated microbial biomass carbon ( $B_C$ ) and organic carbon stock in paddy soils as influenced by soil types and management practices at the long-term experimental fields for manuring and the farmers' fields designated for a monitoring purpose. The  $B_C$  values were affected by soil types and increased by organic matter applications, ranging from 200 to 1,168 mg C/kg. The  $B_C$  values in Brown Lowland soils were higher than those in Gray lowland soils. In contrast, the values of microbial biomass nitrogen ( $B_N$ ) did not show any appreciable difference among the soil types. The  $B_C$  and  $B_N$  values corresponded to 1.1-5.8 and 1.7-5.1% of the respective values of soil organic matter. Similar tendencies were also observed in the farmers' fields for monitoring, the number of the fields being about 40. The average values of soil carbon stocks for the depth of 0-30 cm in lowland soil groups ranged from 47 to 63 Mg C/ha.

## Key Words

Paddy soil, microbial biomass, soil organic carbon, carbon stock, organic matter application.

## Introduction

Shiga prefecture is facing various problems including the issue of water quality in the Lake Biwa, the largest freshwater lake in Japan. It is surrounded by paddy fields, which occupy 92% of the total farmland area in Shiga. Hence, the prefectural government has been promoting environmentally conscious agriculture (ECA) through a direct payment system since 2004. The ECA practice for paddy rice extended to 10,117 ha in 2008, corresponding to 30% of the total rice cultivation area in Shiga, and the ratio was highest in Japan (Shibahara 2009). Furthermore, according to the vision for a sustainable society in Shiga towards 2030, we target to halve the emission of greenhouse gases (GHGs) from the 1990 level. As agricultural management practice has received much interest recently as a source and a sink of GHGs, we have participated in the National Soil Survey Program for monitoring soil carbon content and soil management since 2008 (Leon *et al.* 2009). Soil microorganisms and their activities are at the heart of several vitally important processes such as carbon sequestration and CO<sub>2</sub> release, the formation and destruction of trace GHGs and many transformations within the nitrogen cycle (Powlson 1994). Furthermore, changes in soil microbial biomass measured over relatively short periods can indicate trends in total organic matter content before these can be detected by chemical analyses (Powlson *et al.* 1987). Therefore, we investigated microbial biomass carbon and organic carbon stock in paddy soils as influenced by soil types and management practices at the long-term experimental fields for manuring and the farmers' fields designated for a monitoring purpose.

## Materials and methods

### *Long-term experimental fields*

The long-term field experiment for paddy rice cultivation was continued from 1975 to 2001 at Central Station of Azuchi (Gray lowland soils) and at Western Branch Station of Kosei region (Brown Lowland soils) in Shiga Prefecture Agricultural Technology Promotion Center. Soil samples of the plow layer were collected from the following plots: plot without organic matter application, rice straw compost plot, and rice straw plot. Microbial biomass carbon (C) and nitrogen (N) were determined by the fumigation-extraction method (Inubushi *et al.* 1991; Shibahara and Inubushi 1995, 1997). Several data sets of soil microbial biomass and soil organic matter determined during the last 10 years (the 16th-25th year period after the initiation) were averaged for calculating the balance sheet.

### Farmers' fields for monitoring

The soil survey program was applied to about 40 farmers' paddy fields that are designated for a long-term monitoring purpose in Shiga prefecture. Soil samples for measuring microbial biomass were collected from the plow layer (0 to 15 cm) under submerged conditions in 2001. Soil samples for measuring organic carbon stock were collected from a depth of 0-30 cm in 2008 and were subjected to determination by a dry combustion method and a core sampling method.

## Results and discussion

### Effects of long-term application of organic matter on soil microbial biomass and organic carbon

Microbial biomass C ( $B_C$ ) and N ( $B_N$ ) in paddy soils are shown in Table 1. The values of  $B_C$  were affected by the soil types and increased by organic matter applications, ranging from 200 to 1,168 mg C/kg. The  $B_C$  values in Brown Lowland soils were much higher than those in Gray lowland soils. In contrast, the values of  $B_N$  did not show any appreciable difference among soil types. The  $B_C$  and  $B_N$  values corresponded to 1.1-5.8 and 1.7-5.1% of the respective values of soil organic matter. These differences between the changes in the  $B_C$  and  $B_N$  values affected the  $B_C/B_N$  ratios, indicating that the composition of microbial biomass can be affected by the soil types and organic matter application. Furthermore, lower contents of total soil organic C (T-C) and lower  $B_C/T-C$  values in Brown Lowland soils suggests that a continuous application of organic matter is important to maintain soil organic C level and nitrogen fertility in well-drained soils.

**Table 1. Microbial biomass C and N as influenced by soil types and organic matter application in paddy fields of the long-term manuring experiment.**

Soil group, texture, (Clay content)	Plot	Application rate of organic matter (kg/ha)		Microbial biomass (mg/kg)			Soil organic matter (g/kg)		$B_C/T-C$ (%)	$B_N/T-N$ (%)
		$O_C$	$O_N$	$B_C$	$B_N$	$B_C/B_N$	T-C	T-N		
Gray Lowland soils, CL (24%)	NPK	—	—	200	29.3	6.8	18.7	1.73	1.1	1.7
	NPKC <sub>20</sub>	1,210	61	386	57.7	6.7	23.5	2.05	1.6	2.8
	NPKS <sub>8</sub> (Fp)	2,890	45	349	59.3	5.9	20.9	1.82	1.7	3.3
Brown Lowland soils, L (15%)	NPK	—	—	706	49.1	14.4	15.5	1.63	4.6	3.0
	NPKC <sub>10</sub> S <sub>7</sub>	3,430	77	1,168	94.1	12.4	20.3	2.41	5.8	3.9
	NPKS <sub>7</sub> (Fp)	2,540	33	1,019	95.7	10.6	18.6	1.88	5.5	5.1

NPK, S<sub>7</sub> and S<sub>8</sub> indicate chemical fertilizer and rice straw applied at 7 and 8 (Mg/ha/y), while C<sub>10</sub> and C<sub>20</sub> as rice straw compost applied at 10 and 20 (Mg/ha/y), respectively.

Fp indicates soil improvement material consisting of calcium silicate and fused magnesium phosphate.

$O_C$  and  $O_N$  indicate the average amounts of carbon and nitrogen per ha derived from organic matter application each year, respectively.

$B_C$  and  $B_N$  indicate microbial biomass carbon and nitrogen, respectively.

T-C and T-N indicate total soil organic carbon and nitrogen, respectively.

### Microbial biomass and organic carbon stock in the farmers' paddy fields

Microbial biomass data in the farmers' fields (sampled and measured in 2001) and soil carbon stocks (sampled and measured in 2008) are shown in Table 2. The average values of  $B_C$  in Brown Lowland soils were higher than those in Gray lowland soils and Gley Lowland soils. On the other hand, the average values of total soil organic matter content (T-C, T-N) and clay contents show an opposite tendency. These results are consistent with the changes of the microbial flora in soils and decomposition rates of soil organic matter. Soil carbon stock to the depth of 0-30 cm was much higher in an Andosol field (116 Mg C/ha) than in those of the other soil groups (35–63 Mg C/ha). Although there was only one Andosol sample in this study, similar values and trends have been obtained in the National Soil Survey Program (Leon *et al.* 2009). The average values of soil carbon stock did not show any appreciable difference among the lowland paddy soil groups (i.e., Gley Lowland soils, Gray Lowland soils and Brown Lowland soils) in this study. Further studies are required to estimate soil carbon stocks more accurately and their changes with soil types and manuring practice more quickly by means of microbial biomass measurements.

**Table 2. Microbial biomass and organic carbon stock in various soil types among the farmers' paddy fields designated for monitoring purpose.**

Classification of cultivated soils in Japan 3rd approximation	Soils collected from the plow layer in 2001						Soils collected from a depth of 0-30 cm in 2008	
	Number of sample (n)	$B_C$ (mgC/kg)	$B_N$ (mgN/kg)	T-C (gC/kg)	T-N (gN/kg)	Clay (%)	Number of sample (n)	T-C (Mg C/ha) (average $\pm$ standard deviation)
Lowland Paddy soils	15	745	82	19.2	1.85	18.6	19	46.8 $\pm$ 10.4
Gley Lowland soils	7	651	100	27.9	2.46	25.9	8	62.5 $\pm$ 18.2
Gray Lowland soils	14	664	79	20.9	1.95	19.7	9	50.2 $\pm$ 8.2
Brown Lowland soils	4	1,023	102	21.8	2.10	17.0	3	56.3 $\pm$ 23.1
Gray Upland soils	1	405	51	17.0	1.58	26.2	1	34.78
Andosols	1	1,653	137	58.4	4.36	17.4	1	116

Each value indicates the average for the respective soil group.  $B_C$  and  $B_N$  indicate microbial biomass C and N, respectively.

## Conclusion

Microbial biomass and organic matter status in paddy soils of the long-term manuring experiment and the designated farmers' fields for monitoring were investigated to estimate organic carbon stocks in agricultural lands, especially paddy soils. Microbial biomass C was affected by soil types and increased by organic matter application. Soil carbon stocks for the depth of 0-30 cm in lowland soil groups ranged from 46.8 to 62.5 Mg C/ha. These results are consistent with the changes of the microbial flora in soils and decomposition rates of soil organic matter. Further studies are required to estimate soil carbon stocks more accurately and their changes more quickly.

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# Nitrogen budget of cattle manure compost incorporated into paddy field.

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## Abstract

To estimate the nitrogen (N) budget of cattle manure compost with sawdust (CMC) in a paddy field in the cool climate region of Japan, well-composted  $^{15}\text{N}$ -labeled CMC was applied to a microplot field experiment. Throughout the experimental period of three crop seasons, N from CMC was taken up by rice plants without a marked decline. The percentages of N taken up derived from CMC to applied N as CMC (NUE) were 2–3% for each year. The N from CMC was taken up by rice plants over the entire growth period was 1–2, 2 and 2–3% as NUE at the panicle initiation, heading and maturity stages, respectively. A significant positive linear correlation was found between the cumulative compost N uptake and the number of days transformed to standard temperature (25°C) over the entire experimental period, including the fallow season. The NUE was identical at CMC application rates ranging from 1 to 4 kg/m<sup>2</sup>. Using  $^{15}\text{N}$ -labeled CMC, the results showed that well-composted CMC was a stable N source for rice plants for at least 3 years, regardless of the CMC application rate (ranging from 1 to 4 kg/m<sup>2</sup>) in the cool climate region of Japan. The distribution of CMC N was 7% in the rice plants accumulated over 3 years, 66–69% in the soil and 24–27% was unrecovered at the end of the third crop season.

## Key Words

$^{15}\text{N}$ , paddy field, cattle manure compost, nitrogen use efficiency, rice.

## Introduction

The demand for agricultural produce, including rice, grown using organic materials as a nutrient source is increasing with recognition of the importance of resource recycling farming systems. At the same time, in intensive livestock-farming areas, a great amount of nitrogen (N) is excreted through livestock waste (Ikumo 2003). Estimation of the N balance indicates that surplus excreta N is loaded in some of these areas (Kohyama *et al.* 2003), which necessitates the effective use of livestock waste in areas other than the intensive livestock-farming areas. Thus, the effective use of organic materials, livestock manure compost in particular, in rice farming is anticipated. For the effective use of livestock manure compost in a sustainable recycling system, information about the fate of livestock manure compost N in the paddy field is indispensable. To precisely evaluate the fate of N in organic materials, the use of  $^{15}\text{N}$ -labeled organic materials is a relevant approach (Hood *et al.* 1999; Muñoz *et al.* 2003; Takahashi *et al.* 2000). The objective of this study was to directly evaluate the N budget of cattle manure compost over 3 years, by applying  $^{15}\text{N}$ -labeled compost to a paddy field in the cool climate region of Japan.

## Methods and materials

### Field experiment

The microplot experiment inside a paddy field was conducted from 2000 to 2002 at the National Agricultural Research Center for Tohoku Region (NARCT), Daisen, Akita, Japan (N39°29', E140°30', altitude 30 m). The soil is a fine-textured gray lowland soil (Typic Fluvaquents by Soil Taxonomy) and soil in the plow layer contained 2.35 g/kg total N on a dry-weight basis. A polyvinyl chloride frame (17 cm × 30 cm, height 20 cm) wrapped at the bottom with unwoven cloth was installed into the plow layer (approximately 15 cm depth) inside the paddy field on 22 May 2000. Fresh soil previously collected from the plow layer (6.27 kg dry weight) was passed through a 1-cm sieve and was well mixed with  $^{15}\text{N}$ -labeled cattle manure compost that contained sawdust (CMC). Compound fertilizer was also mixed with the soil. The mixture of soil, CMC and fertilizer was put into the frame on 22 May 2000. The application rates of CMC were 1, 2 and 4 kg/m<sup>2</sup> on a fresh-weight basis, and the rate of compound fertilizer was 8 g/m<sup>2</sup> (as N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O). The  $^{15}\text{N}$ -labeled CMC was made from feces collected from a cow fed with  $^{15}\text{N}$ -labeled corn (*Zea mays* L.). Before composting, sawdust was added to the cattle feces at a rate of approximately 30% to make suitable moisture conditions (Yamamuro 2000). Three replications were set up for the 1 and 4 kg m<sup>-2</sup> CMC treatments. For the 2 kg m<sup>-2</sup> treatment, 21 replicates were initially set up because 18 of 21 replicates were to be eliminated at on-season sampling times. Puddling was carried out on 23 May. On 25 May, 35-day-old rice plant seedlings

(*Oryza sativa* L., cv. Akitakomachi), three seedlings per microplot, were transplanted at the center of each microplot. To the field outside the microplots, only compound fertilizer was applied at a rate of 8 g/m<sup>2</sup> (as N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O), and rice plants were planted at a spacing of 17 cm × 30 cm, the same planting density as the microplot. Plant top samples in the 2kg/m<sup>2</sup> CMC treatment were collected at the panicle initiation stage (10 July) and at the heading stage (3 August) with three replications, respectively. Plant top and soil samples of all treatments were collected at maturity stage (13 September). Plant roots were left in the soil. After sample collection at the maturity stage, the microplots (soil and root in the frame) remained in the ground, up to approximately 15 cm depth, until the next crop season. In 2001 and 2002, only compound fertilizer was applied to the soil of the microplots before crop season; and then similar procedures were carried out.

#### Sample analysis

Total N of the soil and plant samples were determined by Kjeldahl method. The <sup>15</sup>N abundance of these samples was measured using Automated Nitrogen and Carbon Analyzer-Solid and Liquid (ANCA-SL; PDZ Europe, Cheshire, UK). Total N and <sup>15</sup>N abundance of the compost were measured following the same procedure. Water extractable NH<sub>4</sub>-N and NO<sub>3</sub>-N of the compost were measured with an Aquatec 5400 Analyzer (Foss Tecator, Hilleroed, Denmark) after extraction by distilled water in a ratio of compost (fresh weight [FW]) : distilled water of 1:10 for 1 h. Total carbon (C) of the compost was measured with a Vario MAX CN (Elementar Analysensysteme, Hanau, Germany). The biological oxygen consumption of the compost was measured for 50 g of the compost (FW) for 30 min at 35°C using a Compotester (Fujihira Industry, Tokyo, Japan).

#### Calculation of the number of days transformed to standard temperature (25°C)

Using daily mean air temperatures during the experimental period, including the fallow season, the number of days transformed to a standard temperature of 25°C (DTS) was calculated using the following equation, which was obtained from Arrhenius' law (Sugihara *et al.* 1986).

$$DTS = \exp(E_a \times (T_a - T_s) / (R \times T_a \times T_s)) \quad (1)$$

T<sub>a</sub> is the daily mean air temperature (K), T<sub>s</sub> is the standard temperature (298 K), R is a gas constant (1.987 cal/deg/mol) and E<sub>a</sub> is the apparent activation energy (cal/mol). As an E<sub>a</sub> value, 13,800 kcal/mol was used, which was obtained by an incubation experiment of CMC under submerged conditions (Sakai and Yamamoto 1999). The relationship between DTS and N uptake derived from <sup>15</sup>N-labeled CMC (Nd<sub>fc</sub>) in the 2 kg/m<sup>2</sup> CMC treatment was examined using a single regression analysis (SAS Institute 2002).

## Results

#### Maturity of the CMC

The compost contained a small amount of inorganic N and was almost odorless. Biological oxygen consumption was as low as 1 µg/g/min (Table 1). In cases where the biological oxygen consumption of compost is less than 3 µg/g/min, the compost can be considered to be in a stable phase with high maturity (Furuya *et al.* 2003). Therefore, the CMC used in this study can be regarded as highly matured CMC.

**Table 1. Chemical properties and biological oxygen consumption of <sup>15</sup>N-labeled cattle manure compost.**

N (g/kg)	<sup>15</sup> N (atom%)	C (g/kg)	C/N ratio	NH <sub>4</sub> -N (g/kg)	NO <sub>3</sub> -N (g/kg)	Moisture (g/g)	Biological oxygen consumption (µg/g/min)
3.95	2.54	93.0	24	0.15	0.02	0.73	1

Fresh weight basis

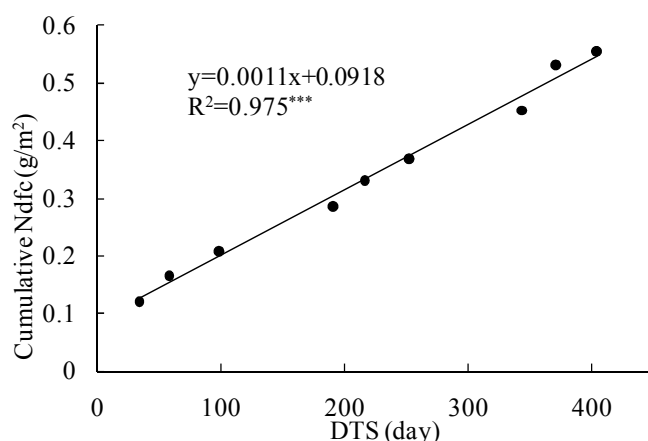
#### Nitrogen use efficiency of CMC for rice plants

The percentages of Nd<sub>fc</sub> (N uptake derived from <sup>15</sup>N-labeled CMC) to applied N as CMC (NUE) are listed in Table 2. The N originated from CMC was taken up by the rice plants in the three crop seasons. The NUE values at the maturity stage were 2–3% for each year, and no marked decline in NUE was observed. Hence, N efficiency of the CMC was stable up to the third crop season from CMC application. In the 2 kg/m<sup>2</sup> CMC treatment, N uptake from CMC continued throughout the growth period for the three crop seasons, and was 1–2, 2 and 2–3% as NUE at the panicle initiation, heading and maturity stages, respectively. Thus, using <sup>15</sup>N-labeled CMC, the results showed that CMC with high maturity was a stable N source for rice plants throughout the entire 3-year growth period. The relationship between DTS (number of days transformed to standard temperature at 25°C) and cumulative Nd<sub>fc</sub> over the 3 years in the 2 kg/m<sup>2</sup> CMC treatment is shown in Figure 1. A significant positive linear correlation was found between the DTS and the cumulative Nd<sub>fc</sub>, suggesting that mineralization of CMC N and its uptake by rice plants depended on the temperature. The NUE at the maturity stage was almost constant regardless of the application rate of CMC, indicating N efficiency per unit weight of the CMC was similar at application rates ranging from 1 to 4 kg/m<sup>2</sup>.

**Table 2. Nitrogen use efficiency (percentage of Ndfc (N uptake originated from  $^{15}\text{N}$ -labeled CMC) to applied N as CMC.**

Year	CMC application rate	Growth stage		
		Panicle initiation	Heading	Maturity
2000	1 kg/m <sup>2</sup>	-	-	2.6 ± 0.6 a
	2 kg/m <sup>2</sup>	1.5 ± 0.3	2.1 ± 0.4	2.6 ± 0.3 a
	4 kg/m <sup>2</sup>	-	-	2.8 ± 0.0 a
2001	1 kg/m <sup>2</sup>	-	-	1.9 ± 0.1 a
	2 kg/m <sup>2</sup>	1.0 ± 0.1	1.6 ± 0.2	2.0 ± 0.2 a
	4 kg/m <sup>2</sup>	-	-	2.0 ± 0.2 a
2002	1 kg/m <sup>2</sup>	-	-	2.4 ± 0.2 a
	2 kg/m <sup>2</sup>	1.1 ± 0.1	2.1 ± 0.1	2.4 ± 0.2 a
	4 kg/m <sup>2</sup>	-	-	2.5 ± 0.1 a

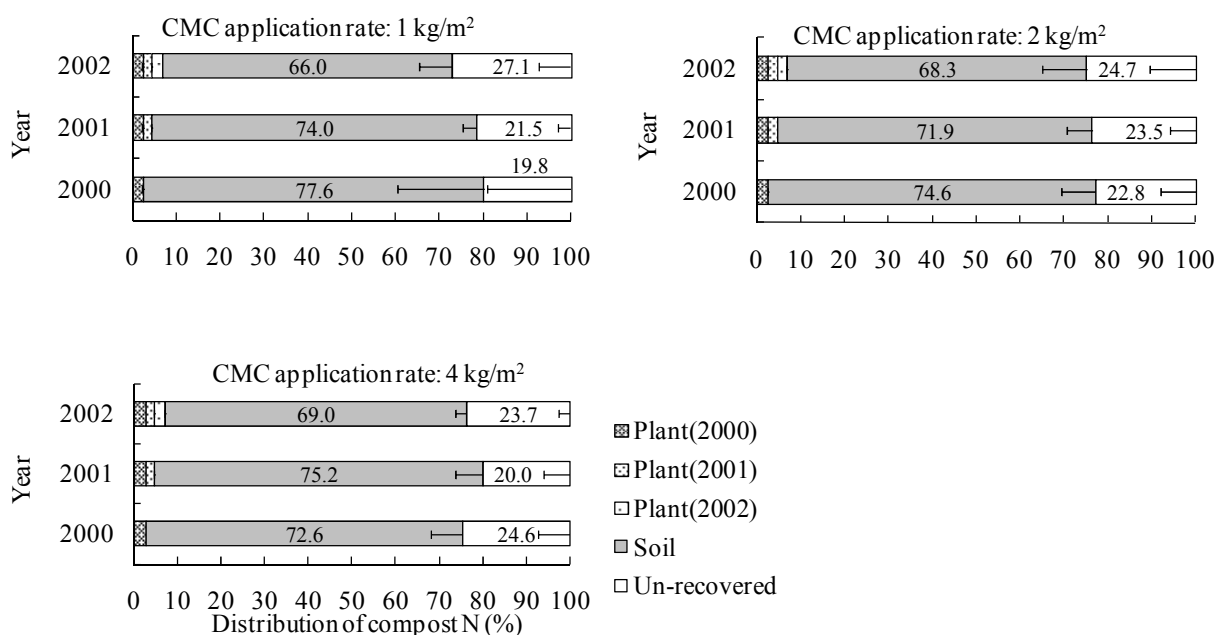
Mean ± SD. Means followed by same letters within a year are not significantly different by Tukey-Kramer's test ( $P < 0.05$ ).



**Figure 1. Relationship between cumulative N uptake originated from  $^{15}\text{N}$ -labeled cattle manure compost (CMC) and number of days transformed at standard temperature (25°C) of the whole experimental period including fallow season in 2 kg/m<sup>2</sup> CMC treatment. Ndfc: N uptake originated from CMC. DTS: number of days transformed at standard temperature (25°C). \*\*\*  $P < 0.001$ .**

#### *Distribution of CMC nitrogen*

The distribution of CMC N was 6.9–7.3% in the rice plants, 66–69% in the soil and 24–27% was unrecovered at the end of the third crop season (Figure 2). The distributions of compost N were similar in all application rates of CMC. The percentage of compost N distributed to the soil did not significantly differ with CMC application rate throughout the experimental period. Greater portion of compost N, approximately 70%, remained in the soil even after the third crop season. This remaining N would continue to be a N source for rice plants. The unrecovered part could represent the loss of the compost N and the N derived from CMC in a substantial part of the rice roots. Nitrogen in the roots was not measured in the present study. However, taking into account the ratios of top to root weight of rice plants, which were around 20, most of the unrecovered part could be ascribed to the loss. The greatest loss was found in the first crop season. Although the tested CMC in this study was highly matured, inherent inorganic N and N in easily decomposable organic matter in the CMC might be lost in the early growth period, before the root system was well developed, in the first crop season. The amount of water percolation in this field was 10 mm/day. Percolation in the field could affect the loss of compost N by leaching. In this study, however, loss through leaching, denitrification and volatilization were not separately evaluated. Hence, the contribution of the loss through leaching to all loss was unclear. A limited number of studies have demonstrated the N distribution of CMC in paddy fields. Among these studies, including the present study, the balances of compost N distribution were different, and a general explanation about N distribution of CMC cannot be made at this time. Further study is needed to clarify relationship among the property of compost, environmental conditions and N budget of the compost.



**Figure 2. Distribution of N originated from <sup>15</sup>N-labeled cattle manure compost (CMC) at the maturity stage. Error bars indicate standard deviation.**

## Conclusion

Using <sup>15</sup>N-labeled CMC, characteristics regarding the N budget of well-composted CMC, which was applied to a paddy field in a cool climate region, appeared to be: (1) N originated from CMC was taken up by rice plants for at least 3 years without marked decline, (2) within a crop season, the N from CMC was taken up over the entire growth period, (3) cumulative N<sub>dfc</sub> linearly increased with DTS for 3 years including the fallow season, (4) at application rates ranging from 1 to 4 kg/m<sup>2</sup>, the NUE of CMC was identical, (5) approximately 70% of compost N remained in the soil even after the third crop season.

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# Origin and chronosequence of paddy soils in China

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## Abstract

The remains of nine ancient rice cultivation sites in the middle and lower reaches of Yangtze River were investigated. The remnants of 24 buried fields, each surrounded by ridges and irrigation ditches were discovered at 100cm depth at the Chuodun site in Kunshan County, China. Many fossil rice grains and utensils, such as pottery vessels and jars, were found in these fields. The fossil rice grains and soil organic matter were carbon dated and these fields were identified as the oldest irrigated rice paddy fields (6280 years BP) known today. It is suggested that the paddy soil developed at this site, at a depth of 100-200 cm was the first paddy soil in China. Based on our work a set of diagnostic criteria for distinguishing ancient paddy fields and soils was proposed. A chronosequence of paddy soils, 50-2000 years BP, developed from the same parent material, under the same ecological conditions and similar cropping systems was found in the south bank of Hangzhou Bay (30°-30.5° N, 121°-122° E). Preliminary results showed that soil fertility and accumulation of organic carbon in the top layer of these paddy soils gradually increased with the extent of cultivation.

## Key Words

Ancient paddy soils, fossil rice grains, rice opal analysis, chronosequence, Yangtze river delta.

## Introduction

Rice is the most important staple food for a large part of the world's human population. China has cultivated rice for more than 5000 years, but the location of the first irrigated paddy field and paddy soil is still being debated in scientific circles. Because of their longevity paddy fields are accepted as a form of sustainable land use, but historically it has been difficult to prove that a field is an ancient paddy because of lack of direct evidence and systematic study. The purpose of this paper is to provide a set of criteria which will enable the ready distinction of relic sites and soils.

## Methods

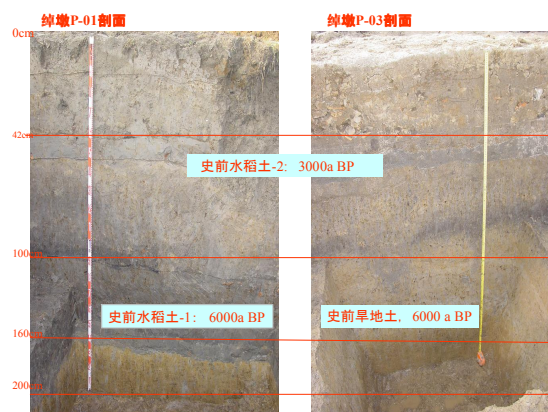
Soil scientists together with archeologists and local agricultural specialists conducted an investigation and excavation of 9 ancient rice cultivation sites in the middle and lower reaches of Yangtze River central China. The traditional methodology used by the Chinese archeologists (Ding 2004) for the excavation of relic sites was adapted for this study. The field survey, description of soil profiles, physical and chemical analyses were carried out according to the Standard Methods (Liu 1996), and the soils were classified according to Chinese Soil Taxonomy (Gong 1999).

## Results

The remnants of 24 buried fields at the 100 cm depth layer in an open area of 300m<sup>2</sup> at the Chuodun relic site (31°24' N, 120°50' E), Kunshan County, Jiangsu Province, China were discovered. Each field is surrounded by ridges and irrigation ditches with water inlets and outlet (Figure 1; Cao *et al.* 2006). Based on the collected pottery, charred rice grains, with rice opal and pollen analysis as well as carbon dating these fields have been identified as the oldest (6280 years BP) irrigated rice fields known (Ding 2004, Cao *et al.* 2006). Accordingly the paddy soils developed here at the depth of 100-200cm (Figure 2) are believed to be the first lowland paddy soils in China. There are two buried ancient paddy soil profiles (3200 years BP and 6280 years BP) which overlapped in the main area (Figure 2, left) and described in Table 1. As the ancient settlers had very simple tools they could construct only very small paddy fields such as these (average area 6.85 m<sup>2</sup>, range 0.4-16m<sup>2</sup>), and had to bury human remains only 15 m from the main area (see skeleton upper right in Figure 1), where only one ancient (3200 years BP) paddy profile (Figure 2 right) had been observed.



**Figure 1. Ancient paddy fields (1), ditches/ponds (2) and human skeleton (3) at the Chuodun relic site.**



**Figure 2. Two buried ancient paddy soils (L) and one buried ancient paddy soil (R) at the Chuodun relic site**

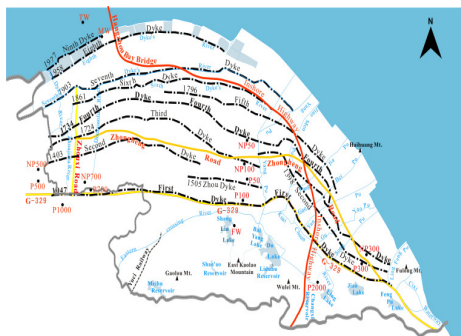
**Table 1 Morphological description of profile-01 at Chuodun relic site**

Depth (cm)	Color	Structure	Texture	Fe & Mn mottles	Bio-remains	Rice opals (no./g)
00-15	10YR3/3	crumb	heavy loam	Many Fe mottles	many rice straw and roots	19476
15-22	10YR4/2	lumpy	heavy loam	Brownish mottles	some rice straw and roots	17093
22-42	10YR4/3	lumpy	light clay	Some Fe & Mn Conc.	a little straw and root	14147
42-57	10YR3/1	block	heavy loam	Silt film/many Fe&Mn Conc.	some Pottery pieces	25271
57-75	5YR4/1	non	light clay	Some Fe & Mn Concretion	a pottery piece	11477
75-100	5YR3/1	non	light clay	non		3542
100-116	10YR2/1	lumpy	heavy loam	Silt film/many Fe&Mn Conc	many charred grain	105159
116-130	10YR4/1	lumpy	heavy loam	Silt film/many	roots and leaves	64007
130-150	7.5YR4/1	lumpy	light clay	Fe mott. & Fe&Mn Conc	Hollow butt	17327
150-160	10YR5/1	block	light loam	Clayish Mott. & some Conc		19678
16-174	10YR5/1	non	light clay	non		0
174-200	10YR4/6	block	light clay	non		0

As part of our studies, we developed a set of diagnostic criteria for distinguishing ancient paddy fields and soils (Cao 2008). These are; the fields/soils should (1) contain more than 50 fossil rice grains per m<sup>2</sup> of area or more than 5000 rice opals/g of soil; (2) be surrounded by bunds and ditches, and contain irrigation equipment and water sources for irrigation, (3) have clear boundaries with depletion or accumulation of clay, iron and other materials in the profile, (4) have specific pollen diagrams in the surface layer of the buried field and a specific magnetic susceptibility change in the profile, and (5) have specific <sup>13</sup>C NMR spectra of soil organic matter in the surface layer of the buried field. If the first and second criteria are met then the opened site can be considered as a buried ancient paddy field. Once a buried profile has met the third to the fifth criteria then it can be diagnosed as an irrigated paddy soil profile.

Although it is accepted that irrigated rice is a sustainable land use type, historically there is little direct evidence. A chronosequence of paddy soils was found in the south bank of Hangzhou Bay, located in Yuyao and Cixi counties (30°-30.5° N, 121°-122° E) of Zhejiang Province China. Between 1074 and 1993, 10-11 sea dikes were continuously constructed to create new farm land from coastal wetland. Consequently a chronosequence of paddy soils 50 to 2000 years old (Figure 3) was gradually formed. These paddy soils have developed from the same parent materials (coastal sediments), under the same ecological conditions and with the similar cropping systems (irrigated rice in summer and upland crops in winter). The fertility of the top soil layers of this chronosequence gradually increased with time of cultivation without the addition of chemical fertilizer (Figure 4 and Table 2).

The organic carbon concentration of the surface layer of these paddy soils gradually increased with time of cultivation to 700 years; since then there was little change (Table 2). The results suggest that organic carbon in the top layer of the paddy soils reached its ecological balance after 700 years. However, the dissolved organic carbon continually moving down and accumulated in the lower horizons (Table 3). The results show that paddy soil is an effective carbon pool even after it has been used for 700 to 2000 years.



**Figure 3. Coastal sediments and dikes on the layer South bank of Hangzhou Bay.**



**Figure 4. Natural soil fertility of the top of 50 to 2000 year old paddy soils.**

**Table 2. Soil fertility characteristics of the top layers of 50 to 2000 year old paddy soils.**

Age (Years )	pH	TOC (g/kg)	Available P (mg/kg)	Available K
50	7.87	14.39	21.32	210.0
500	7.37	15.48	22.36	157.3
700	7.37	18.23	17.64	125.3
1000	6.93	19.95	26.67	234.7
2000	6.52	20.20	35.71	211.3

**Table 3. Variation in organic carbon density in profiles of 50 to 2000 year old paddy soils.**

Age (Years)	D 0-20cm (g/m <sup>2</sup> )	D 0-40cm (g/m <sup>2</sup> )	D 0-80cm (g/m <sup>2</sup> )	VR (%)
50	3866.66	4785.30	6013.49	289.62
100	4402.01	5129.53	6525.86	267.36
500	2700.35	3380.74	4431.54	221.73
700	3438.21	4495.79	6131.30	174.89
1000	3432.49	4815.84	6904.87	130.53
2000	3502.08	5694.64	9333.90	56.48

## Conclusions

1. A total of 24 buried fields of various sizes were located at 100cm depth in the Chuodun relic site, Kunshan, China and identified as the oldest (6280 years BP) irrigated paddy fields known. The results suggest that the paddy soil developed there at a depth of 100-200cm was the first paddy soil in China.
2. A set of diagnostic criteria for characterizing ancient paddy fields and ancient paddy soils was proposed.
3. A chronosequence of 50 to 2000 year old paddy soils developed from the same parent materials (coastal sediments), under similar ecological conditions and cropping systems were found in the south bank of Hangzhou Bay. Preliminary results showed that fertility and organic carbon accumulation in the top soil layer and the density of dissolved organic carbon in the lower layers of the soil profile gradually increased with time of cultivation,

## Acknowledgements

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# Water balance in dry seeded and puddled transplanted rice in Punjab, India

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## Abstract

Rice systems that increase production using less water are urgently needed, especially in North West India where ground water is over-exploited. Replacing the traditional puddled transplanted rice (PTR) with dry seeded rice (DSR) is often proposed as a means of increasing water productivity and saving water. A field study was conducted in Punjab, India, in 2008 to investigate components of the water balance and water productivity in DSR and PTR with different irrigation schedules. Irrigation scheduling was based on different thresholds of soil water tension (SWT). The input water productivity ( $WP_{I+R}$ ) of rice was significantly higher in DSR irrigated at 20 kPa (0.71 g/kg) than in all other treatments followed by PTR at 20 kPa (0.50 g/kg). The differences in  $WP_{I+R}$  between PTR and DSR at 20 kPa were largely due to reduced seepage. However, deep drainage beyond 0.6 m soil depth was higher in DSR, presumably due to the absence of hard pan in the non-puddled system. There was no significant difference in evapotranspiration (~600 mm) between DSR and PTR when irrigation was scheduled on the basis of SWT.

## Key Words

Irrigation schedule, Soil matric potential, Evapotranspiration, Water productivity.

## Introduction

Over exploitation of ground water resources is a major threat to the sustainability of the traditional system of puddled transplanted rice (PTR) production in Indo-Gangetic Plains. Rodell *et al.* (2009) reported a groundwater depletion rate of  $4.0 \pm 1.0$  cm/yr over the North West Indian states of Rajasthan, Punjab and Haryana. The alarming rate of ground water decline is forcing researchers and farmers to modify their present production system to a water-wise system. The conventional method of rice growing is not only a water-guzzling but also cumbersome and laborious. In Punjab, where agriculture is highly dependent upon migrant labour, labour scarcity, especially for transplanting is another major concern in the existing production system. Unscheduled electricity power cuts due to high demand in both industrial and farming sectors also adversely affect farming practices. Both these factors will increasingly result in delayed transplanting, which may reduce rice yield and delay sowing of wheat.

Studies in the north-west IGP have shown that rice can be grown successfully dry seeded into non-puddled soils, with or without prior cultivation. Dry seeded rice (DSR) provides a gateway for advancing crop establishment to make better use of early season rainfall, and facilitates an increase in crop intensification in rice based systems (Tuong *et al.* 2000). However, DSR needs to be sown earlier, so the field is exposed to higher evaporative demand for a much longer period than a puddled transplanted field. However, Bouman (2001) claimed the potential water savings at the field level in upland rice due to less evaporation since there is no permanent ponded water layer, and the amount of water used for puddling is eliminated altogether. However, many researchers have found similar input water productivity ( $WP_{I+R}$ ) for both dry seeded and conventional PTR (Cabangon *et al.* 2002; Singh *et al.* 2005; Bhushan *et al.* 2007; Choudhury *et al.* 2007).

The effect of DSR versus PTR on water use and water productivity is not well understood, and will depend on many factors including climate, soil type and crop management (especially irrigation management). There is need to study the water balance components viz. irrigation water inputs, deep drainage, runoff and evapotranspiration (ET), so as to objectively compare the water use and water productivity of DSR with that of PTR. The present study aimed to do this for a range of irrigation schedules.

## Materials and methods

### Experimental site

The study was carried out at the research farm (30°54' N, 75°98' E, 247 m ASL) of Punjab Agricultural University, Ludhiana, India during 2008. The soil is clay loam with 0.5% organic carbon content. The climate of the region is sub-tropical with hot, wet summers and cool dry winters.

### *Treatments*

The experiment was laid out in 4 replicates in a split plot design with 2 establishment method (EM - dry-seeding and puddled-transplanting) in main plots and 4 irrigation scheduling (IS - daily, and irrigation when the soil water tension (SWT) at 20 cm increased to 20, 40 or 70 kPa) treatments in sub-plots. The daily irrigated PTR treatment was continuously flooded; however this was not the case in the daily DSR because of the high infiltration rate. The daily treatments were topped up to 50 mm standing water depth. The alternate wetting and drying (AWD) tension-based treatments received 50 mm at each irrigation. Sub-plot size was 9 m x 7 m. The sub-plots were bounded by earthen bunds with a plastic lining to a depth of 0.5 m.

The seedling nursery for transplanting and the DSR plots were sown with the medium duration (144 d) variety 'PAU-201' on 9<sup>th</sup> June 2008. The DSR was sown with a hand plough at 40 kg seed/ha (establishment density 150-180 plants/m<sup>2</sup>) and a row spacing of 20 cm. Transplanting was on 5th July in rows 20 cm apart and plant to plant spacing of 15 cm. For the first 45 d after sowing, the DSR was irrigated to maintain soil water tension at 10-15 kPa at 10 cm soil depth to avoid stress during crop establishment. All PTR treatments were continuously flooded for the first 15 days after transplanting prior to commencement of the irrigation scheduling treatments.

### *Crop management*

Recommended fertilizer and other crop management practices were followed, and control of weeds, pests and diseases was excellent. The crop was harvested at approximately 15-20 % grain moisture content on 21<sup>st</sup> October (daily irrigated treatments) and 3<sup>rd</sup> November (other treatments). Grain and straw yield were determined on an area of 5 m x 5 m in the middle of the plots.

### *Water balance measurements*

All components of the water balance were measured directly or indirectly, except for ET which was estimated from the water balance equation:

$$I + R = R_{\text{off}} + S + D + \Delta\text{SWC} + \text{ET} \quad (1)$$

The volume of irrigation (I) water volume applied to each plot was measured with a Woltman<sup>®</sup> helical turbine meter. Rainfall (R) was measured using an automatic rain gauge installed in the centre of the experimental site. Runoff ( $R_{\text{off}}$ ) occurred on heavy rainfall days and was calculated from the amount of rainfall and the difference between the height of the bunds and the water depth prior to the rainfall. The tritium injection method of Munnich (1968) was used to calculate drainage (D) below the root zone. Tritiated water was injected at 60 cm depth at the time of sowing DSR or transplanting. The change in soil water content ( $\Delta\text{SWC}$ ) was calculated from bulk density and gravimetric SWC to a depth of 60 cm before establishment and after harvest.

### *Input water productivity*

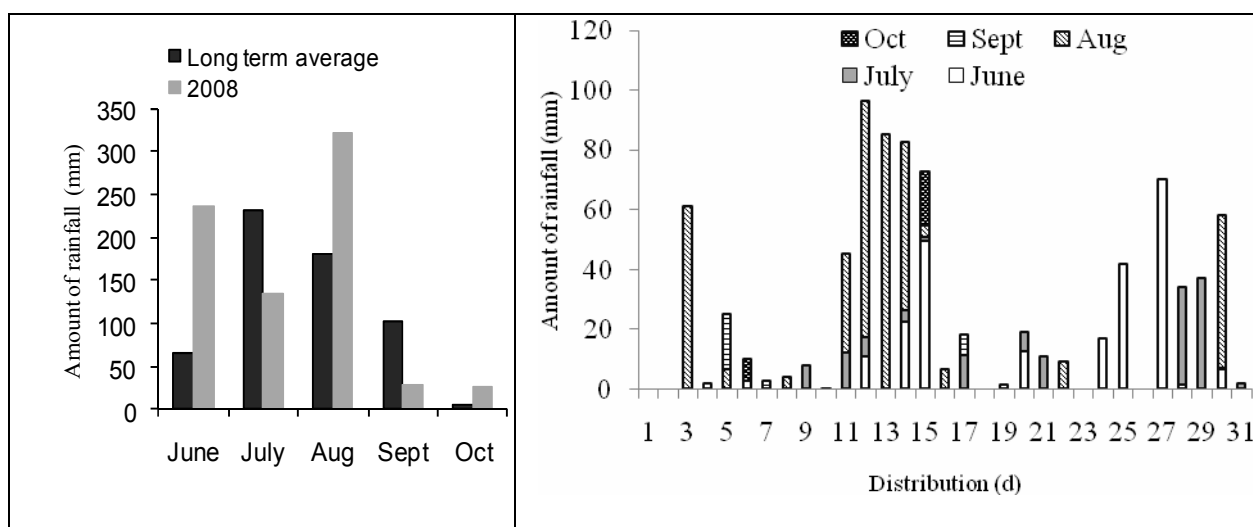
Input water productivity ( $\text{WP}_{\text{I+R}}$ ) was computed as the ratio of grain yield (14% moisture) to the total water input (irrigation + rainfall) and expressed as g/kg.

## **Results**

The total amount of rainfall was 28 % higher than the long term average (586 mm) and well distributed, especially in the first 3 months of crop growth (Figure 1)

There was a significant interaction between EM and IS on the amount of irrigation (Table 1). Irrigation water input was similar in the daily irrigated PTR and DSR, but significantly lower in all AWD DSR treatments than in AWD PTR. At 20 kPa this resulted in a 485 mm (53%) irrigation water saving. Within EM, irrigation amounts in all AWD treatments were similar because of the good rainfall distribution. The difference in the irrigation water was use mainly due to recommended practice of continuous flooding in PTR for first 15 days after transplanting.

Deep drainage below 0.6 m in DSR was significantly higher than in PTR. This was probably because the soil was not puddled and because of the longer duration of DSR in the field, and some large rains between sowing and the time of transplanting. Deep drainage was significantly higher with daily irrigation (662 mm) than with AWD (175-227 mm).



**Figure 1. The amount (mm) and distribution (d) of the rainfall during rice season in 2008.**

**Table 1. Water balance components in dry seeded and puddled transplanted rice.**

Establishment method (EM)	Irrigation scheduling (IS)	Water Balance Components (mm)							WP <sub>I+R</sub> (g/ kg)
		Irrigation	Rainfall	Deep drainage	Seepage	Runoff	ΔSWC	ET	
				below 0.6 m			(0-0.6 m)		
PTR	Daily	2093	566	559	823	489	32.1	756	0.28
	20 kPa	1010	566	170	580	161	2.1	663	0.50
	40 kPa	960	566	141	580	161	1.5	643	0.44
	70 kPa	960	566	147	580	161	1.5	636	0.35
	Mean	1255	566	254	641	243	9	675	0.39
DSR	Daily	2212	822	982	491	585	54.8	921	0.24
	20 kPa	475	822	284	227	165	10.7	610	0.71
	40 kPa	425	822	273	227	165	4.4	577	0.40
	70 kPa	358	822	203	227	165	4.4	580	0.35
	Mean	868	822	436	293	270	19	672	0.43
Mean of PTR and DSR	Daily	2153	694	771	657	537	43	839	0.26
	20 kPa	743	694	227	404	163	6	637	0.61
	40 kPa	693	694	207	404	163	3	610	0.42
	70 kPa	659	694	175	404	163	3	608	0.35
LSD (p≤0.05)									
EM		231.0	-	30.1	60.6	ns	5.68	ns	ns
IS		133.0	-	24.4	34.5	29.4	2.44	132	0.069
EM × IS		204.0	-	33.2	ns	41.6	3.45	127.0	0.088

The heavy rains early in the season damaged the bunds and dislodged the plastic, and the development of a high earthworm population resulted in large seepage losses up to 52 DAS. The amount of water loss by seepage was significantly higher in PTR (641 mm) because all plots were continuously flooded during this period. Seepage was significantly higher (657 mm) with daily irrigation than AWD (404 mm).

Runoff occurred during some high rainfall events, and was significantly higher (by 70%) with daily irrigation (537 mm) than AWD (163 mm). Changes in stored soil water were small relative to other components of water balance. The increase in stored soil water was significantly higher in daily irrigated DSR (55 mm) than daily irrigated PTR (32 mm), probably because of the rains before transplanting. Change in SWC of the AWD treatments was very small (2–11 mm).

There was a significant interaction between EM and IS on ET. Evapotranspiration in daily irrigated DSR (921 mm) was significantly higher than in any other treatment, probably because of the higher biomass production under DSR (17.0 t/ha) than in PTR (15.8 t/ha), and because DSR was exposed to a longer evaporative demand period than PTR (26 d between sowing DSR and transplanting). However ET in DSR and PTR was similar under all AWD conditions (608–663 mm).

There was no interaction effect of EM and IS on grain yield. PTR produced significantly higher grain yield as compared to DSR. Yields with daily irrigation and irrigation at 20 kPa were similar, but there was a significant decline in yield with irrigation at higher tensions. There was a significant interaction between EM and IS on  $WP_{I+R}$ , which was highest in DSR with irrigation at 20 kPa (0.71 g/kg) followed by PTR irrigated at 20 kPa (0.5 g/kg) and least in daily irrigated PTR (0.28 g/kg). The difference in  $WP_{I+R}$  between DSR and PTR at 20 kPa was largely due to reduced seepage losses and despite higher deep drainage losses in DSR, while ET and runoff were similar. At higher water tension (40 and 70 kPa) there was no significant difference  $WP_{I+R}$  between DSR and PTR because the reduced irrigation amount in DSR was offset by a similar reduction in grain yield in DSR.

## Conclusion

In a year of above average and relatively well distributed rainfall, alternate wetting and drying resulted in very large irrigation water savings in both PTR and DSR, mainly due to large reductions in deep drainage, underbund seepage and runoff. Irrigation at 20 kPa soil water tension reduced irrigation input by 50% in DSR compared with PTR, while maintaining yield. As a result, input water productivity was highest (0.71 g/kg) in DSR irrigated at 20 kPa tension, and least in continuously flooded PTR (0.28 g/kg). Further evaluation is needed under a range of environmental conditions to help develop irrigation scheduling guidelines for DSR and to assess the likely nature and amount of water savings from adoption of DSR.

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# Winter cover crops increase soil carbon and nitrogen cycling processes and microbial functional diversity

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## Abstract

Winter cover crops are not only one of effective agricultural management practices to control weeds but also can improve soil fertility, resulting in increasing crop productions. Up to now, however, little is known about information on how much of soil soluble organic carbon (C) incorporates into the soils applied with winter cover crops, which is a prerequisite to design strategies that improve C sequestration in agricultural ecosystems. The aims of this study were to: (1) assess the effects of winter cover crops on soluble organic carbon (SOC) pools using different extraction methods (KCl extractable organic C; microbial biomass) and microbial community functional diversity, and (2) quantify how much of the potentially mineralizable organic C pools ( $C_0$ ) incorporates into the soils and associated half-life of SOC remaining under seven cover crops and nil-crop control (CK) in temperate agricultural soils of southern Australia. Cover crop treatments are cereal rye, wheat, saia oats, vetch, field peas, mustard and the mixture of cereal rye and vetch. Results showed that the CK treatment had higher soil moisture content and lower soluble organic nitrogen (SON) compared to the cover crop treatments. Among the cover crop treatments, there was significantly higher SON in the wheat, oats and vetch treatments than in the other treatments. The oats treatment had the highest amount of cumulative  $CO_2$ -C than any other treatments over one-month incubation experiment. An exponential regression approach for C mineralization was used to estimate  $C_0$  and soil samples under the cover crops can be divided into four groups depending on  $C_0$ . The principal component analysis of the MicroResp<sup>TM</sup> profiles showed that the CK treatment was significantly different from the cover crop treatments. The cover crop treatments with wheat, vetch and peas as well as mustard form a cluster which was significantly different from the other clusters. In addition, the vetch, field peas and mustard treatments showed higher Shannon diversity H and Evenness (E) and Simpson diversity H compared to the other cover crop treatments with the lowest Shannon H and E at CK. In conclusion, overall, the vetch and field peas as well as wheat winter cover crop may be better management practices for agricultural ecosystems in southern Australia.

## Introduction

Many of studies have shown that cover crops are not only an effective agricultural management practice to control weed but also provide many services to agro-ecosystems, such as decreasing erosion, improving soil nutrient retention and building soil organic matter (SOM) (Carrera *et al.* 2007; Sainju *et al.* 2008). Most of these studies, however, have dealt with either single or a few cover crops and there are very few studies on an overall evaluation of the role of cover crops in the C sequestration by combining soil labile C and N pools and microbial functional diversity and cover crop biomass. Soil organic matter is an important ecosystem property and regarded as widely acknowledged indicator for soil quality (Chen *et al.* 2004). However, it can take many years to detect differences in SOM under different types of management. Labile fractions of SOM like SOC and SON are very important because they control ecosystem productivity in the short term and are more sensitive to management practices (Huang *et al.* 2008). A number of techniques, including KCl extraction and microbial biomass measurement by fumigation, have developed to fractionate labile C pools from SOM. Labile C pools comprise the easiest available sources of energy for microorganisms and therefore, it is useful to study when analyzing the soil microbial community functional diversity using MicroResp<sup>TM</sup> which has been shown to be more discriminatory than Biolog (Campbell *et al.* 2003). As SOC has been regarded as an indicator for soil quality and its decomposition is a function of different factors, an understanding of C mineralization in soils applied with cover crops is a prerequisite for predicting contributions of these SOC pools to global  $CO_2$  balance and helping to develop strategies to sequester more SOC into these soils and to maintain soil functions. The aims of this study were to (1) assess the effects of winter cover crops on SOC pools using different extraction methods and microbial community functional diversity, and (2) quantify how much of the potentially mineralizable organic carbon pools ( $C_0$ ) incorporate in soils and associated half-life of SOC remaining under seven cover crops compared to the nil-crop control (CK).

## Methods and materials

The research was conducted in a full factorial design with seven cover crops, i.e., cereal rye, wheat, saia oats, vetch, field peas, mustard and mixture of cereal rye and vetch (designated as mixture) and the nil-crop control (CK) with three replicates in Wagga Wagga Institute in southern Australia. The area of each plot is 40 m<sup>2</sup> with a rowing space of 22 cm between plots. Seeds of cover crops were broadcast on 29<sup>th</sup> May 2009, with the sowing rate of 80 kg/ha for rye, 80 kg/ha for wheat, 80 kg/ha for oats, 50 kg/ha for vetch, 100 kg/ha for peas, 5 kg/ha for mustard and 45 (cereal rye) and 40 (vetch) kg/ha for the mixture. Fertilizer applied on all plots on the same day of broadcasting was diammonia phosphate at a rate of 80 kg/ha, including 20 kg N, 18 kg P and 2-3 kg S/ha. Soil samples were collected on 9<sup>th</sup> Oct 2009 after the cover crops were harvested. Five cores were taken to a depth of 10 cm in each plot. The concentration of inorganic N was measured a Lachat Quickchem automated ion analyzer. SOC and SON were extracted using KCl extraction method, described by Huang *et al.* (2008). Microbial biomass was measured by chloroform fumigation-extraction method (Chen *et al.* 2004). Soil microbial community functional diversity was measured using the MicroResp<sup>TM</sup> system with the application of 16 C sources (Campbell *et al.* 2003). Plant aboveground biomass was measured by clear cutting at the ground level at a 1-m<sup>2</sup> quadrat placed in each plot. All ANOVA, regression and t-test analyses were performed using SPSS 11.0 software (SPSS Inc., USA). The microbial community diversities based on MicroResp<sup>TM</sup> were submitted to principal component analysis (PCA). To estimate the potentially mineralizable C<sub>0</sub> and first-order rate constant (k), the non-linear regression approach for N mineralization of Smith *et al.* (1980) was used:  $C_m = C_0 * (1 - \exp^{-kt})$ , where C<sub>m</sub> is the organic C mineralized (mg/kg) at a specific time (t).

## Results

Table 1 shows some basic soil properties in soils under the cover crops. The CK treatment had significantly higher soil moisture content and lower pH and SON compared to the cover crop treatments. There were not significant differences in microbial biomass C (MBC) and N (MBN) among the treatments. Among the cover crops, SON was significantly higher in wheat, oats and vetch treatments than in others. Cover crop aboveground biomass was significantly lower in the field peas than in the other treatments. The cover crop with oats had the highest amount of cumulative CO<sub>2</sub>-C evolved with the lowest amount in the CK treatment and the intermediate in the other treatments (Figure 1). By comparison of C<sub>0</sub> in the treatments, statistical analyses showed that the soils under the cover crops had significantly higher ability to release SOC as CO<sub>2</sub> compared to the CK. Four distinct groups of soils were distinguished for C<sub>0</sub> values: Group 1 included the cover crops with oats and field peas; Group 2 included the cover crops with wheat, vetch and mustard; Group 3 included the cover crops with cereal rye and mixture; Group 4 included the CK. The decomposition constant (k) and the time required to mineralize one-half of the potentially mineralizable C (t<sub>1/2</sub>) are shown in Table 3. The average basal respiration measured using MicroResp<sup>TM</sup> ranged from 0.02 to 0.30 ug CO<sub>2</sub>-C/g/h (Figure 2). All substrates induced respiration (SIR) above the basal respiration (water only). SIR for L-arginine, citric acid, malic acid, oxalic acid, D-fructose and D-glucose was significantly higher than the other C sources. The CK showed higher utilization capacities for L-arginine, citric acid and L-lysine. Based on MicroResp<sup>TM</sup>, Shannon diversity index H and Evenness (E) we found H and E were significantly higher in the cover crops than in the CK (Table 4). Among the cover crops, the cover crops with vetch, field peas and mustard showed significantly higher Shannon H and E compared to the other treatments. In addition, Simpson diversity index H showed the similar trend with the highest H in the vetch and field peas treatments. The principal component analysis of the MicroResp<sup>TM</sup> profiles showed that CK treatment is significantly different from the cover crop treatments. The cover crops with wheat, vetch and peas as well as mustard form a cluster which was significantly different from the other clusters (Figure 3).

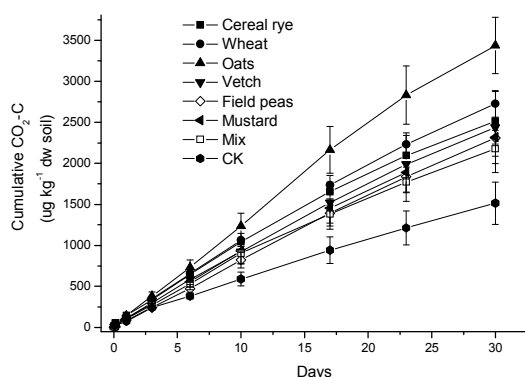
## Conclusion

With overall evaluation of SOC and SON pools, C<sub>0</sub>, cover crop production as well as microbial functional diversity index and evenness, the vetch and field peas as well as wheat winter cover crop may be better management practices for agricultural ecosystems in southern Australia.

**Table 1. Selected soil properties under different winter cover crops in southern Australia.**

Treatments	pH	Soil moisture	SOC (mg/kg)	SON (mg/kg)	MBC (mg/kg)	MBN (mg/kg)
Cereal rye	5.0±0.05a	5.7±0.6b	688±31ab	9.7±0.2b	426.5±22.4	78.9±4.7
wheat	5.1±0.06a	6.4±0.7b	730±8a	14.2±0.6a	430.9±31.0	84.0±11.1
Saia oats	5.0±0.05a	5.3±0.4b	710±38a	12.2±0.8ab	392.5±18.1	71.3±5.0
Vetch	5.1±0.08a	5.5±0.6b	710±36a	14.6±0.4a	401.8±73.0	65.3±12.1
Field peas	5.0±0.07a	7.3±0.9b	718±28a	10.5±0.3b	462.7±26.4	69.6±7.5
Mustard	5.0±0.02a	5.9±0.1b	688±24ab	11.9±0.4ab	425.1±8.0	70.9±6.2
Mixture	5.1±0.02a	5.5±0.5b	674±29ab	10.9±0.8b	455.3±22.7	74.0±10.7
CK	4.6±0.05b	11.1±0.4a	586±46b	1.4±0.3c	394.0±15.7	69.4±8.8

Data are mean ± S.E. (n=3). Means within a column followed by the same letter are not different at the 5% level of significance by t-test.

**Figure 1. Cumulative CO<sub>2</sub>-C evolved from soils under cover crops in southern Australia.****Table 3. Comparison of calculated potentially mineralizable organic C pools (C<sub>0</sub>) and first order rate constants (k) and half life of C remaining in soils under cover crops in southern Australia.**

	C <sub>0</sub>	k	R <sup>2</sup>	Half-life (day)
Cereal rye	4.93c	0.02395	0.99	29
Wheat	6.61b	0.0178	0.99	39
Oats	12.59a	0.01081	0.99	64
Vetch	6.66b	0.01526	0.99	45
Field Peas	12.62a	0.00677	0.99	102
Mustard	5.59b	0.01786	0.99	39
Mix	4.74c	0.02042	0.99	34
CK	3.45d	0.01902	0.99	36

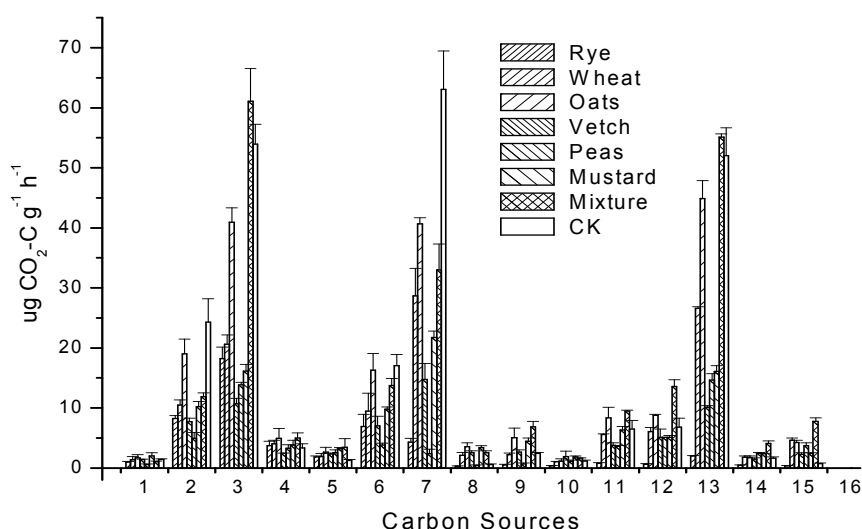
**Table 2. The properties and aboveground biomass of different cover crops in southern Australia.**

Treatments	C (%)	N (%)	Biomass (kg/ha)	C:N ratio
Cereal rye	40.9±1.8	1.67±0.22c	3857±79ab	25.36±1.73b
wheat	41.1±0.4	1.35±0.12c	4858±72a	30.84±1.79a
Saia oats	42.5±0.1	2.38±0.22b	3930±34ab	18.21±0.84c
Vetch	41.5±3.5	3.14±0.23a	4871±27a	13.20±0.18d
Field peas	41.4±0.7	1.77±0.25c	4858±26a	24.18±1.16b
Mustard	43.1±0.5	2.47±0.18b	3323±25b	17.63±1.51b
Mixture	42.7±0.1	1.78±0.23c	4294±45ab	24.88±1.37b

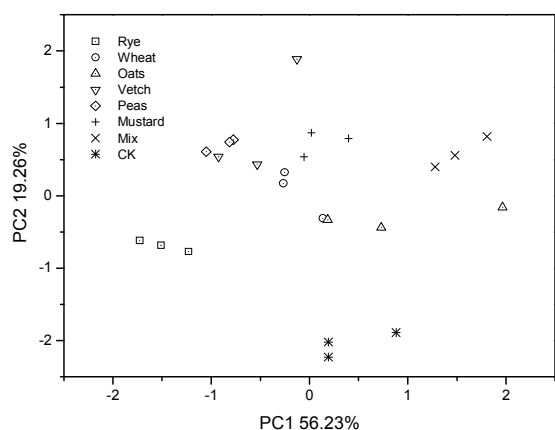
Data are mean ± S.E. (n=3). Means within a column followed by the same letter are not different at the 5% level of significance by t-test.

**Table 4. Shannon diversity index H and Evenness as well as Simpson diversity index H based on the calculation of microbial community functional diversity using MicroResp<sup>TM</sup> under different cover crops in southern Australia.**

Treatments	Shannon H	Evenness	Simpson H
Cereal rye	2.13±0.02c	0.53±0.01cd	0.72±0.02d
Wheat	2.28±0.04b	0.61±0.03b	0.84±0.01b
Oats	2.21±0.04b	0.57±0.03bc	0.83±0.01b
Vetch	2.61±0.05a	0.85±0.04a	0.87±0.01a
Field Peas	2.48±0.05a	0.74±0.04a	0.83±0.01b
Mustard	2.52±0.02a	0.78±0.02a	0.87±0.01a
Mix	2.17±0.04b	0.55±0.02d	0.82±0.01bc
CK	1.93±0.02d	0.43±0.01e	0.80±0.01c



**Figure 2. Respirometric evolution of carbon dioxide after 6 h with or without the addition of 15 different C sources for soils under different cover crops in southern Australia. The numbers from 1 to 16 correspond to represent L-alanine, L-arginine, citric acid, D-galactose, amino butyric acid, malic acid, oxalic acid, 3,4-OH benzoic acid, L-arabinose, L-cysteine, D-fructose, D-glucose, L-lysine, N-acetyl glucosamine, D-trehalose, water, respectively.**



**Figure 3. Ordination plot of the first two canonical axes produced by principal component analysis (PCA) based on MicroResp™ C source utilization profiles for soils under different cover crops.**

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# Deficit Irrigation an option to mitigate Arsenic load in Rice Grain

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## Abstract

Field trial was carried out during 2008 to assess impact of irrigation on arsenic status of soil, leaf and grain of rice and water use efficiency of the crop. Four levels of irrigation i) continuous ponding (CP); ii) intermittent ponding (IP); iii) saturation and iv) aerobic were imposed to the crop during 15 to 45 days after transplanting (DAT). Rest of the period, irrespective of regimes crop was exposed to CP. Highest grain yield recorded under IP. Arsenic load in soil, leaf and grain attained highest value under CP and decreased in the order of IP>Saturated>Aerobic regimes. Impact of irrigation regimes on variation in total arsenic load was maximum in soil (80.5%) followed by leaf (40%) and grain (18%). Arsenic added to the rice field through contaminated water poses strong relationship with total arsenic status of soil, leaf and grain. Water use efficiency attained highest value under aerobic regime and closely followed by IP.

## Introduction

Rice-rice is the main cropping sequence of Bengal delta, which covers one state (West Bengal) of India and major part of Bangladesh. Notably higher (55 – 80%) grain yield over the rainy season rice motivate the farmers to cultivate rice (summer rice) in post winter season (February to March) under irrigated condition. Farmers irrigate 1200 to 1400 mm water to meet up the higher (3-5.5 mm/day) evapotranspiration demand during its growing period and 70% of this meets up by ground water. Arsenic is a known carcinogen and as per World Health Organization (WHO) it is highly toxic to human health when its concentration in water goes above 50 µg /l. At present ground water arsenic concentration of 2/3<sup>rd</sup> land area of Bangladesh and 1/3<sup>rd</sup> land area of West Bengal is reported above the WHO critical level. This contamination puts at least 100 million people at risk of cancer and other arsenic related diseases. Irrigate rice with polluted water resulted in increase of arsenic status in different parts of the crop (Meharg 2004). Thus million of people who are living out side the arsenic contaminated area also consume the toxic arsenic every day by eating rice grown in the contaminated area. Besides, amount of fresh water and share of irrigation in it, is showing a declining trend against time. In contrast lowest (20 – 30 kg/ha/mm) value of water productivity has been reported for rice (Zwart and Bastiaanssen 2004). Curtail in amount of irrigation water at stress allowable stage of the crop can enhance crop water productivity without notable decrease in yield (Sarkar 2001). Considering these a farmer's field study was planned to assess the role of different levels of deficit irrigation on reduction of arsenic load in rice grain and improve its water productivity.

## Methods

The experiment was conducted on farmers field located (23°02' N, longitude 88°35' E; altitude of 8.8 m amsl) in an arsenic affected village of West Bengal, India during 2008. The soil of the field was of silty loam type (Aeric Haplaquept). Four levels of irrigation were: i) Continuous ponding (CP), followed by the farmers of the locality and was the control treatment; ii) Intermittent ponding (IP) irrigation was given when soil matric potential ( $\Psi_m$ ) at 20 cm depth reaches to – 0.02 M Pa after disappearance of ponded water; iii) Saturation where 0 to 50 cm soil profile was maintained at saturation state and iv) Aerobic where irrigation was given when  $\Psi_m$  at 50 cm depth reaches to – 0.05 M Pa to recharge 0 to 50cm soil at field capacity ( $(\Psi_m = - 0.03\text{M Pa})$  level. Treatment ii, iii and iv were imposed during 15 to 45 DAT. There after till 80 DAT under all the regimes crop was exposed to CP. Depth of irrigation under CP was  $4 \pm 1$  cm. The experiment was laid out in a randomized block design with three replications. Rice variety Gontera Selection 3 was taken as the test crop. Last irrigation was given on 80 DAT. Water, plant and soil samples were digested with tri acid mixture, filtered and processed for estimation of total arsenic by using an atomic absorption spectrophotometer (Perkin-Elmer make, model: Analyst – 200) coupled with FIAS-400 hydride generator.

## Calculations

The depth of irrigation for IP was calculated by the equation proposed by Chaudhary (1997) as:

$$D_i = (\theta_s - \theta_i) D_r + D_s \quad (1)$$

Where,  $D_i$  is, depth of irrigation (mm);  $D_r$  is, depth of root zone (mm);  $D_s$  is, depth of submergence (mm) in

this study  $D_s$  was 50 mm;  $\theta_s$  is the average volumetric moisture content (AVMC,  $\text{m}^3 \text{m}^{-3}$ ) of the root zone at saturation and  $\theta_i$  is the AVMC ( $\text{m}^3 \text{m}^{-3}$ ) at the time of irrigation. The term  $(\theta_s - \theta_i)$  gives the volume of water required to raise the water content of a unit volume of soil to saturation.

## Results

Imposition of deficit irrigation during 15 to 45 DAT decreased the amount of arsenic added to rice field over CP by 40 to 130% and 19 to 62% respectively at 45 DAT and at harvest (Table 1). Under all the regimes adoption of a common irrigation level (CP) from 46 to 80 DAT was responsible for the reduction in variation of arsenic at harvest over 45 DAT. Irrespective of the study period total arsenic status in soil, leaf and grain attained highest value under CP and decreased in the order of IP>Saturated>Aerobic. On 45 DAT, under different regimes variation in total arsenic status of soil and leaf was 81 and 45% respectively. At harvest the same for soil and leaf reduced to 80 and 35%. In case of rice grain the variation was 18%. Amount of arsenic added to rice ecosystem through contaminated water shows strong relationship with total arsenic status of the soil and rice leaf (Figure 1 and 2). The relationship was stronger with soil than that of the leaf. Impact of irrigation on total arsenic status of soil and leaf was more prominent at 45DAT than at harvest. Grain yield also possess a good relationship with arsenic added through contaminated water (Figure 3). Arsenic added through irrigation showed strongest relationship ( $R^2 = 0.94$ ) against soil arsenic status. Data presented in table 1 and the  $R^2$  value of figure 4 revealed that study site soil act as a good sink on arresting the amount of arsenic added to the soil and reduces the transport of arsenic to rice grain.

Difference in amount of water irrigated under four regimes significantly varied the grain yield (Table 2). Under IP regime application of 200 mm less water increased grain yield by 4%. This results support the hypothesis of negative impact of arsenic on grain yield of rice. Exposures of a water loving crop like rice to higher degree of water stress (Saturated and aerobic regimes) have negative impact on various physiological processes, which was well reflected in grain yield status. Present study shows that plant can recover the moderate degree of water stress (IP) if it imposed at the stress allowable stage. Water use efficiency (WUE) attained the highest value under aerobic regime followed by IP. Magnitude of WUE decreased by 5.11, 10.78 and 36.56% respectively under IP, saturated and CP regimes over aerobic condition.

## Conclusions

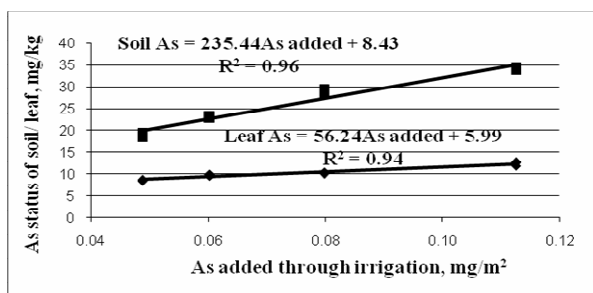
Considering degree of arsenic pollution, yield and water use efficiency as well as easiness in operation intermittent ponding can be adopted by the farmers of the arsenic contaminated area in place of continuous ponding. Soil acts as a good sink in arresting arsenic towards its transport to rice leaf and grain.

**Table 1. Effect of irrigation levels on total arsenic status of soil and plant parts at different crop stages**

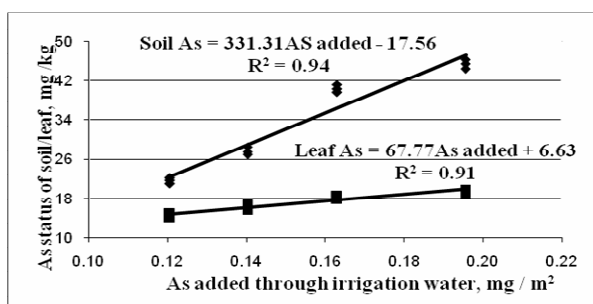
Irrigation levels	At 45 Days after transplanting			At harvest			
	Arsenic added, $\text{mg/m}^2$ soil	Total arsenic, $\text{mg/kg}$		Arsenic added, $\text{mg/m}^2$ soil	Total arsenic, $\text{mg/kg}$		
		Soil	Leaf		Soil	Leaf	Grain
CP	0.11	34.05	12.34	0.20	44.34	19.49	0.98
IP	0.08	28.85	10.28	0.16	40.40	18.29	0.93
Saturated	0.06	23.06	9.79	0.14	27.62	16.35	0.90
Aerobic	0.05	18.75	8.51	0.12	24.61	14.38	0.83

**Table 2. Amount of water irrigated, grain yield (GY) and water use efficiency (WUE) under varying levels of irrigation**

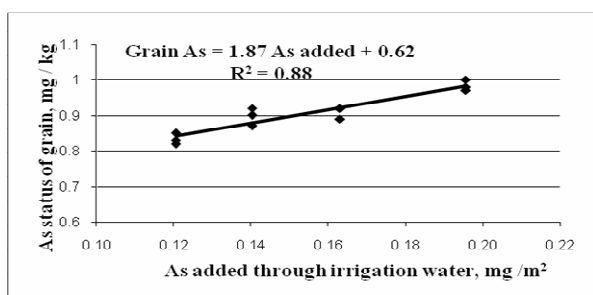
Irrigation levels	Water irrigated, mm				GY, t/ha	WUE, $\text{kg/ha/mm}$
	0-15 DAT	16-45 DAT	45-80 DAT	Total		
CP	170	520	510	1200	4.33	3.61
IP	170	320	510	1000	4.69	4.69
SAT	170	200	510	880	3.92	4.45
ARB	170	130	510	740	3.65	4.93
LSD ( $P=0.05$ )					0.33	



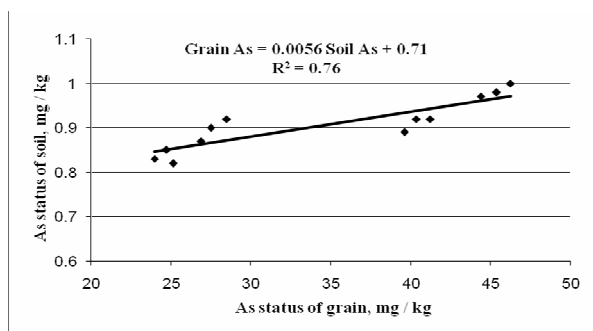
**Figure1. Relationship between arsenic added through irrigation with total arsenic status of soil and rice leaf at 45 days after transplanting.**



**Figure2. Relationship between arsenic added through irrigation with total arsenic status of soil and rice leaf at harvest.**



**Figure 3. Relationship between arsenic added through irrigation with total arsenic status of grain.**



**Figure 4. Relationship between total arsenic status of soil with total arsenic status of grain.**

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# Effect of Water Management on Zinc Concentration in Rice Grains

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## Abstract

The study was conducted to evaluate the effect of water management on grain Zn concentration and uptake. A pot experiment was conducted at University of the Philippines Los Baños, Soils and Agro-Ecosystems Division screen house from June to October, 2005. Results revealed that continuous flooding ( $W_1$ ) increased grain yields of MS13 and IR72 in Buguey possibly due to increased availability of nutrients brought about by shallow submergence of the soil as reflected on grain NPK uptake. In Alimodian, where inherent fertility status is lower, alternately wetting and drying ( $W_2$ ) resulted in increased NPK uptake and grain yield. IR72 was more sensitive to changes in water management and soil fertility status. In contrast, the water requirement of MS13 can be reduced without significantly reducing its yield.

Grain Zn concentration and uptake were also influenced by the ability of the soil to supply Zn, rice variety and water management. Zinc concentration in grains was significantly higher in Alimodian with higher level of HCl-extractable Zn. In both soils, grain Zn concentration of MS13 was higher compared to IR72. This indicates that water management had no effect on grain Zn concentration in MS13 when soil Zn concentration is below the critical level set for rice. This confirms the inherent capacity of MS13 as very efficient in absorbing and loading Zn in its grains. By partly drying ( $W_2$ ) the Buguey soil during the growing period of IR72 significantly increased grain Zn concentration.

## Key Words

Zinc concentration in grains, water management in rice, continuous flooding, alternate wetting and drying, soil Zn,

## Introduction

The International Zinc Nutrition Consultative Group (IZiNCG) estimated that as much as one third of the world's population is at risk from inadequate zinc intake. Although the human body needs tiny amounts of vitamins and minerals for normal growth and development, their absence costs lives and causes disabilities and mental impairment ([www. IZiNCG.org](http://www.IZiNCG.org)). Even a small increase in micronutrient content in rice would have a significant impact on the human health since cereal - based diets are the major source of nutrients for the majority of the world's population especially poor Asians. Both the nutrient content, especially iron and zinc, of the cereal grains and its bioavailability to the people consuming them are of fundamental importance. FAO believed that the nutrient content of rice can be improved substantially by using both traditional selective plant breeding and new biotechnology approaches.

In response, a concerted effort of plant breeders resulted in a significant progress in rice – a high yielding, disease resistant, iron enriched, and aromatic variety was identified (IR 68144-3B-2-2-3) and released as Maligaya Special #13 (MS-13). The said variety is able to tolerate low concentration of available iron and zinc in the soil and it is more efficient in taking up and loading these trace elements into the grains (Alloway, 2004).

There are reports, however, that some rice varieties that are efficient in loading high Fe and Zn densities in their grain do not consistently manifest the same characteristics in other conditions or areas. This shows that plant uptake of Zn depends not only upon species, age, or parts of the plant, but also on the amount that is available in the soil (Chlopecka *et al.* 1996).

The breeder's efforts in rice biofortification must then be complemented by identifying water management practices that can influence Zn availability in soils and subsequently Zn concentration in rice grains so that biofortification program would be more cost-effective and sustainable endeavor. This study was conducted to identify the water management practice that would lead to increased availability of soil Zn and subsequently to (1) increased grain Zn concentration; (2) investigate the relationship between soil Zn

concentration and Zn uptake; (3) determine the influence of soil properties on grain yield, Zn concentration and uptake;

## Methods

### *Soil sampling and preparation*

Soils used in the experiment were identified to have low zinc content through Minus One Element Technique (MOET) of previous studies conducted by Descalsota *et al.* (2002). Soil samples were collected from the top 0 - 20 cm layer of lowland rice fields at Guisguis, Sariaya (Buguey soil) and Bamban, Tagkawayan (Alimodian soil), Quezon province. The collected soil samples were kept submerged for about four weeks with alternate mixing to maintain the reduced condition of the soils. Minus one element technique (MOET) was conducted on the soils for further nutrient content evaluation.

### *Soil Taxonomic Classification*

Buguey soil series was classified as mixed, isohyperthermic, typic Ustipsamments. This soil was developed from the recent alluvium and recent coastal deposits. Alimodian soil series was classified as fine montmorillonitic, isohyperthermic Udorthentic Chromudert. The soil was formed from the unconsolidated sediments from sandstone-shale materials.

### *Pot Set-up*

10 kg of Buguey soil and 12 kg Alimodian soil were placed in the plastic containers. Holes were made at the bottom of the plastic pails and placed with rubber stoppers with rubber and glass tubing to facilitate draining of water. Properly marked sticks were placed in each pot to serve as guide for the level of floodwater and electrodes to monitor the soil redox potential (Eh) (Plate 1).

### *Soil and Plant Analysis*

Soil analysis for pH, organic matter (OM), total N, available P, exchangeable K, and available Zn were performed before planting. Plant tissue concentration of N, P, K and Zn content in dehulled brown grains from all treatments were analyzed. N, P, K and Zn uptake in grain and straw were also determined.



**Plate 1. Pot experimental setup**

### *Treatments*

The different treatment combinations were replicated three times and were laid out in completely randomized design (CRD) (Plate 1a). The factors employed for the treatment combinations were as follows:

#### **Factor 1:**

##### **Rice varieties (V)**

- V<sub>1</sub> - MS 13  
(IR68144-3B-2-2-3)
- V<sub>2</sub> - IR72

#### **Factor 2: Lowland**

##### **Rice soils (S)**

- S<sub>1</sub>—Buguey loamy sand  
(Sariaya)
- S<sub>2</sub>—Alimodian silty  
clayloam (Tagkawayan)

#### **Factor 3: Water Management (W)**

- W<sub>1</sub> - Continuously flooded (the pots were irrigated to at least 5 cm of standing water 5 days after transplanting (5 DAT) and were kept flooded until 2 weeks before harvest (WBH))
- W<sub>2</sub> - Alternate wetting and drying cycle (the pots were irrigated to at least 5 cm water starting 5 DAT and allowed to dry until the soil starts to crack before it was again flooded. The cycle was repeated until 2 WBH)
- W<sub>3</sub> - Drying at maximum tillering stage (the pots were irrigated to at least 5 cm water starting 5 DAT then drained to saturated at tillering stage (no. of days depended on the variety used) then it was kept flooded again until 2 WBH)

### *Fertilizer Application*

A total of 150 – 90 – 60 kg N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O/ha were applied. Nitrogen fertilizer was applied in three splits (30 % basal, 50 % topdressed 5-7 days before panicle initiation (DBPI) and 20 % topdressed 5-7 days before flowering). All of P was applied as basal and K was split twice (50 % basal and 50 % 5-7 DBPI). Management of different moisture regimes were strictly followed as specified in the treatments using distilled water to avoid introduction of zinc from other sources.

## Results

### Soil physico-chemical properties

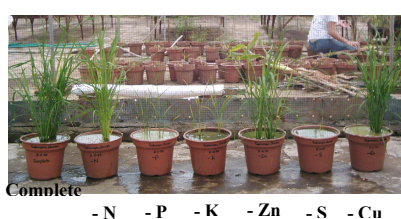
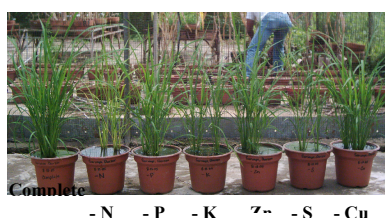
Table 2 lists the initial physicochemical characteristics of the soils used in the experiment.

**Table 2. Physicochemical characteristics of the two soils used in the preliminary experiment.**

SOIL PROPERTIES	BUGUEY SOIL	ALIMODIAN SOIL
Particle size distribution		
% sand	44	17
% silt	38	47
% clay	19	36
Texture	sandy clay loam	silty clay loam
pH	6.65	5.15
Organic matter content (%)	6.89	2.57
Nitrogen (%)	0.34	0.25
Available Phosphorus (mg/kg)	59.48	4.98
Exchangeable Potassium (mg/kg)	0.288	0.129
0.05N HCl Extractable Zinc (mg/kg)	0.360	1.07

### Minus – One Element Technique (MOET)

Minus one element technique is a simple and easy method of assessing soil fertility status. Plate 2 shows the result of the MOET conducted in Buguey. The growth of the rice plants indicated that nitrogen (N), potassium (K) and sulfur (S) are deficient in the soil, but sufficient in phosphorus (P), copper (Cu) and zinc (Zn). Fertilizers with N K S must be applied as corrective measures for normal growth and development and for a better yield.



**Plate 2. MOET test in Buguey soil. Plate 3. MOET test in Alimodian soil.**

In Alimodian, almost all nutrients were very deficient except Cu (Plate 3). Nutrient deficiencies were so evident and physiological and agronomical characteristics of the plants were greatly affected. Without proper and balanced fertilization, this may lead to yield reduction or even yield loss.

### Grain Yield and NPK Uptake

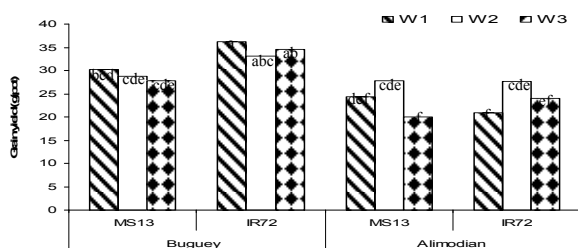
Grain yield was markedly influenced by water management (W) and soil (S) (Figure 1). The effect of water management however differed between the two soils as indicated by a significant interaction between W x S. In Buguey, higher grain yields were obtained under continuous flooding ( $W_1$ ). This trend was in agreement with the results of a field experiment conducted by Agarwal *et al.* (1985) wherein continuous shallow submergence ( $5 \pm 2$  cm) throughout plant life proved to be the best water regime. The differences in grain yield were however not statistically significant indicating that reducing water supply did not significantly reduce grain yield.

In Alimodian, alternate wetting and drying cycle ( $W_2$ ) resulted in the highest grain yield. Subjecting this soil to  $W_2$  resulted in increased solubility and availability of nutrients. The increased grain yield was related to the apparent increase in nitrogen (N) uptake of both varieties as shown in Figure 2. It was evidently showed that the effect of drying was governed by such factors like soil type and inherent fertility of the soil.

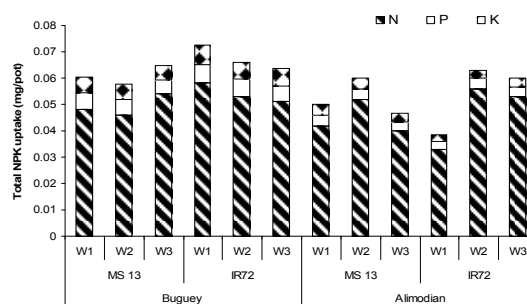
The two varieties also differed in their response to the treatments imposed. A significant interaction between variety (V) x soil (S) was observed. In Buguey, IR72 consistently yielded higher than MS13 under all water management practices. An average yield of  $34.58 \text{ g pot}^{-1}$  was recorded for IR72 as compared to  $28.85 \text{ g pot}^{-1}$  for MS13. The grain yield of the two varieties however did not differ markedly in Alimodian. An average yield of  $24.05 \text{ g pot}^{-1}$  and  $24.20 \text{ g pot}^{-1}$  was recorded for IR72 and MS13, respectively.

IR72 appeared to be more influenced by water management and soil fertility status. The grain yield of MS13 was only significantly reduced when the soil was drained at maximum tillering stage ( $W_3$ ).

Total NPK uptake in grains followed the same trend as grain yield (Figure 2). In Buguey,  $W_1$  resulted in higher NPK uptake followed by  $W_3$  then by  $W_2$ . In Alimodian, total NPK uptake was in the order:  $W_2 > W_3 > W_1$ .



**Figure 1.** Effect of water management on grain yield of two rice varieties grown on two soils.



**Figure 2.** Effect of water management on total NPK uptake in grains of two rice varieties grown on two soils.

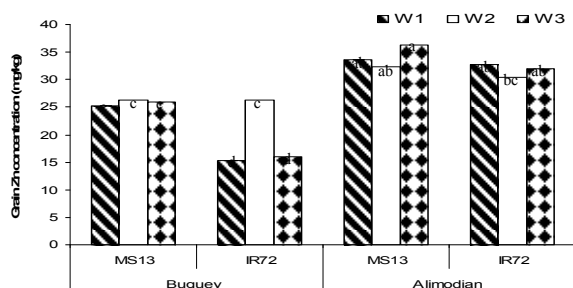
### Zinc Concentration and Uptake in Grains

Grain Zn concentration in both varieties was significantly higher in Alimodian, regardless of water management (Figure 3). Alimodian has a higher ability to supply Zn as it was found to contain about three times as much 0.05N HCl – extractable Zn (1.07 mg/kg) than Buguey (0.360 mg/kg).

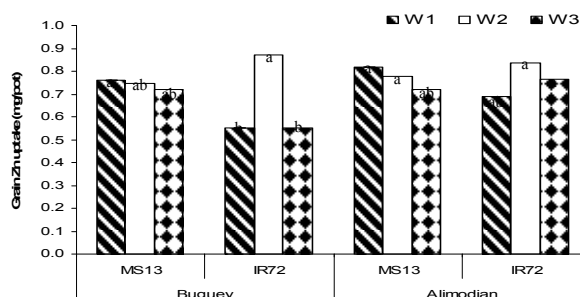
In both soils, Zn concentration in the grains of MS13 was higher than in IR72. In Buguey, the average grain Zn concentration was 25.89 mg/kg for MS13 and 19.22 mg/kg for IR72. In Alimodian, MS13 had a grain Zn concentration of 34.11 mg/kg while IR72 had 31.67 mg/kg. The ability of MS13 to absorb Zn from the soil and load it to its grains is very evident in Buguey which has a much lower Zn content.

Zinc concentration in grains of both varieties grown in Buguey followed the order:  $W_2 > W_3 > W_1$  while in Alimodian highest was in  $W_3$  followed by  $W_1$  and then  $W_2$ .

Significant interaction between  $W \times V$  was also observed. Grain Zn concentration and uptake (Figure 4) of MS13 was not significantly influenced by water management. For IR72, however,  $W_2$  significantly increased Zn concentration and uptake in grains, particularly in Buguey where Zn content is below the critical level.



**Figure 3.** Grain Zn concentration of two rice varieties in two soils as influenced by water management.



**Figure 4.** Grain Zn uptake of two rice varieties in two soils as influenced by water management.

### Conclusion

Grain Zn concentration and uptake of MS13 was not affected by water management practice particularly under low Zn levels as in the case of Buguey. A high yielding variety (IR72) was more sensitive to changes in water management and soil fertility status. Its capacity to absorb more Zn and load it to its grain as compared to IR72 became very evident when the Zn level of the soil is below the critical level set for rice. MS13, which has been introduced for the biofortification program, can be grown under different water management practices in Zn-deficient soils particularly on soils with high inherent fertility status without significant effect on its agronomic performance and mostly the Zn concentration in grains.

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# Quantitative and qualitative responses of rice genotypes (*Oryza Sativa*) to salinity levels of drained water

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## Abstract

High quality water deficiency and consecutive drought obligate use of uncommon water. Therefore evaluation of tolerance potential of crops to stress is necessary. However, responses of four rice varieties (G28, Rahmatabadi, Hassani, and Shahpasand) to four salinity levels water for irrigated (control, 4, 6, and 8dS/m) investigated. This study was carried out in split experimental with based on RCBD with 3 replications for 3 years. Results showed that by increasing of salinity levels, mean weight of shoot dry matter, grain yield of paddy, and stubble weight decreased from 12100 to 4206, 4067 to 861, and 8036 to 3345 kg/ha respectively. Also, by increasing of salinity levels, plant height, total number of tiller, number of fruitful tiller, number of full grain per cluster, and kernel weight decreased from 88 to 65, 38 to 18, 24 to 9, 44 to 22, and 24.5 to 19.5 respectively. But number of hollow grain per cluster increased from 21 to 33. Responses of varieties was different, that G28 with foliage dry matter weight, grain yield, stubble weight, plant height, number of full and hollow grain per cluster, 9739, 3926, 5814 kg/ha, 84.1 cm, 48, and 13 was the best respectively. On the contrary, this indices for Hassani cultivar with 6080, 1376, 4704 kg/ha, 66.1 cm, 17, and 32 was the worst. Every four varieties with a view to quality affected from salinity, because gelatinization temperature was over the range of 3-5, and gel consistency below the range of 41-60. But Amylose percent was at the range of acceptable (20-25). In the meantime, G28 with gelatinization temperature of 5.6 and gel consistency of 30.5 is the best, but these indices at Hassani cultivar with 6.5 and 29.3 respectively is the worst. Amount of elements (N, P, K, Na, Cl, and Mg) of grain with 2.2, 0.46, 0.58, 0.123, 0.393, and 0.171 percent is the most at 8dS/m treatment respectively. By increasing of salinity amount of Mn decreased from 77 to 44, but Zn increased from 20 to 27mg/kg.

## Introduction

Salinity is a major threat to crop productivity in Iran especially south of Iran. Deficiency of high quality water, and to appear of consecutive drought use of uncommon water is unavoidable. Salinity by reducing turgor, expanding tissues and osmotic regulation seriously effected on plant growth (Heidari and Mirzaie 2006).

Tolerance of plants isn't a constant characteristic and maybe at different stage of growth for various species will be different (Linghe and Shannon 2000). Percent of embryo settlement, dry weight of plant, and rice yield, significantly decreased with increasing of salinity (6). Rice plant is moderately tolerance to salinity at seedling stage and the embryo stage is high sensitive, and again at vegetative growth stage is resistant, also at pollination stage become sensitive, and at ripening stage to become exceedingly tolerance (Lang *et al.* 2001a, Lang *et al.* 2001b, and Moradi 2002). By increasing salinity levels (1-16 dS/m) germination, plant settlement, wet and dry matter of shoot and root decreased and Na and Cl of leaf 5 and 3 times of control treatment at 16 dS/m respectively, and K of leaf about 40 percent decreased, but Ca and Mg isn't affected (Shannon *et al.* 1998).

Salinity significantly effected on grain yield, plant settlement, and grain weight per plant and per cluster, number of spikelet per cluster, but no significantly effect on compression of cluster, kernel weight, shoots weight (Zeng and Shannon 2000). Gain *et al.* (2004) showed that plant height and plant biomass reduced significantly from 7.81dS/m salinity. Salinity tolerance rice genotypes had better expression of morphological characters than the salinity susceptible under saline condition, and salinity above 3dS/m sharply reduced all growth characters (Razzaque *et al.* 2009). In spite of spread study on rice and salinity, there aren't enough quantities information's about rice cultivars threshold of responses. Therefore aim of this study is to search the possibility of some salinity tolerance cultivar for successfully rice producing with brackish water.

## Materials and methods

In this research responses of 4 varieties of G28, Rahmatabadi, Hassani and shahpasand to 4 levels of irrigation water salinity including: control (no brackish), 4, 6 and 8 dS/m at split plot experimental with based on randomized complete block design with 3 replication, during 3 years was investigated.

Experimental plots besieged with galvanize sheet iron and salinity treatments to exert by mixture and composed of drained water with irrigation water. Salinity of water under rice plant controlled with portable EC meter. Growth, yield and yield component including: total shoot weight, grain yield, stubble weight, plant height, cluster length, total number of tiller, fruitful tillers, sterile tillers, number of full and hollow grain per cluster, kernel weight, amount of nutrient elements in grain including: N, P, K, Ca, Mg, Na, Cl, Fe, Zn, Cu, Mn, and quality characteristics of rice including Amylase percent, gelatinization temperature, and gel consistency measured and investigated.

## Results and Discussion

Mean soil test result at different experimental plots showed in table1.

**Table1. Mean soil test result at different experimental plots**

pH	SP %	EC	K(mg/kg)	P(mg/kg)	OC %	N%	Sand%	Silt%	Clay%	TNV %
7.58	49	1.63	313	12.5	1.01	0.1	12.9	42	45.1	39

Mean comparison of paddy yield and yield components under affected of salinity level treatments, showed in table 2.

**Table2. Mean comparison of salinity levels affected on yield and yield component of rice plant.**

Treatments	Total foliage weight per ha	Grain yield per ha	Stubble weight per ha	plant height	Total number of tiller	Number of fruitful tiller	Number of full grain per cluster	Number of hollow grain per cluster	Kernel weight
Control	12100A**	4067A**	8036A**	88A**	38A**	24A**	44A**	21B**	24.5A**
4 dS/m	9851B**	2859B**	6992A**	83B**	38A**	23B**	42B**	22B**	23.2A**
6 dS/m	7531C**	2209B**	5321B**	79B**	25B**	15B**	35B**	25B**	23.1B**
8 dS/m	4206D**	861.3C**	3345C**	65C**	18C**	9C**	22C**	33A**	19.5C**

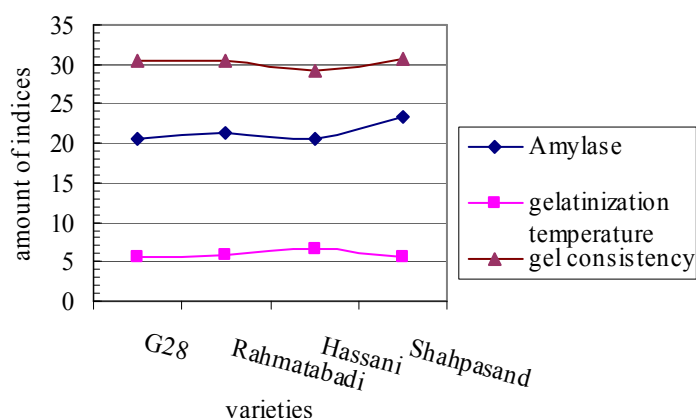
Table2 showed that by increasing of salinity levels mean foliage weight, paddy grain yield, stubble weight, plant height, total number of tiller, number of fruitful tiller, number of full grain per cluster, and kernel weight decreased, but number of hollow grain increased, that indicating bad effect of salinity on pollination of paddy. Maximum yield of paddy obtained from no saline water and its minimum obtained from 8dS/m treatment, and salinity levels of 4 and 6dS/m was intermediate.

Mean comparison paddy yield and yield components of different varieties of under affected of salinity treatments showed in table 3.

**Table3. Mean comparison of different rice varieties yield and yield components under salinity effects.**

Treatments	Total weight of foliage per ha	Grain yield per ha	Stubble weight per ha	plant height	Number of full grain per cluster	Number of hollow grain per cluster	Kernel weight
G28	9739A**	3926A**	5814BC**	84.1A**	48A**	13B**	19D**
Rahmatabadi	9014A**	2827B**	6187AB**	81.4A**	39B**	25A**	20.9C**
Hassani	6080B**	1376C**	4704C**	66.1B**	17C**	32A**	24.1B**
Shahpasand	8857A**	1868C**	6989A**	83.6A**	40AB**	31A**	26.3A**

Minimum total foliage weight, grain yield of paddy, stubble weight, plant height, and full grain per plant, obtained from Hassani. On the contrary maximum total foliage weight, grain yield, plant height, and number of full grain per cluster, obtained from G28 cultivar. After G28 cultivar, maximum grain yield obtained from Rahmatabadi, whereas Hassani and Shahpasand cultivars produced minimum grain yield. Maximum stubble yield obtained from Sahpasand, because this cultivar had good vegetative growth but its grain didn't become full. Minimum plant height and stubble yield obtained from Hassani because it's had low tolerance to salinity. Maximum full grain, minimum hollow grain, and maximum grain yield obtained from G28, and Shahpasand, Rahmatabadi, and Hassani had the less full grain, but for propose of hollow grain their had similar group.



**Figure1. Quality indices of different varieties under effected of salinity levels.**

Suitable range of amylase percent is 20 to 25, and below from this amount, cooked rice is much sticky, and above from that rice is much hard. In this study amount of amylase of even 4 varieties was in the suitable range. But suitable range of gelatinization temperature index is 3-5, amounts of below from 3 means that threshold of converted to gel is upper and to take more boil, but number of more than 5 indicated rice at less degree of temperature converted to gel. In this research maximum amount of this index is 6.53 obtained from Hassani and amounts of 5.89, 5.63 and 5.56 obtained from Rahmatabadi, Shahpasand and G28 respectively.

Suitable range of gel consistency is 41-60, that number of below from 41 is indicating that rice after cooking will be hard. This index divided three stage of hard, medium, and soft. Analysis result of this research showed that amount of gel consistency for 4 varieties was below from 41. Therefore, rice after cooking will be hard. Nearest variety to suitable range was G28, and the worst variety was Hassani.

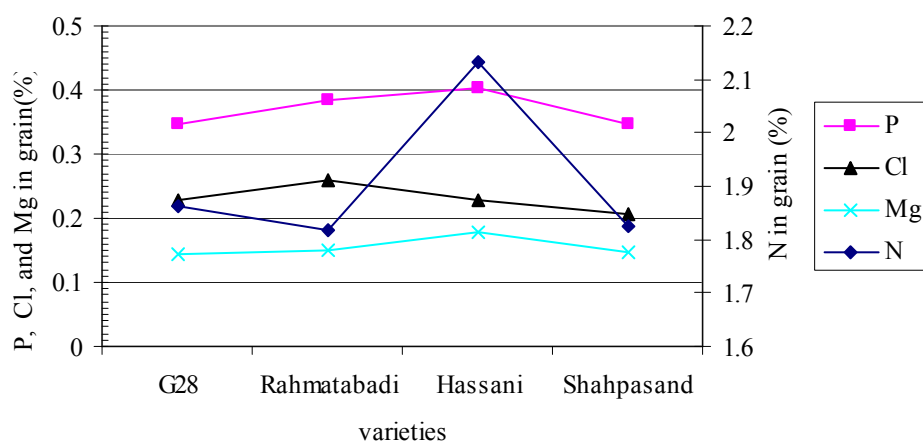
Grain nutrient elements were measured and its mean amounts under affected of salinity levels on different varieties investigated.

**Table4. Mean comparison of salinity levels affect on amounts of nitrogen (N), Phosphorus (P), Potassium (K), Sodium (Na), Chlorite (Cl), Magnesium (Mg), Manganese (Mn), and Zinc (Zn).**

Treatments	N (%)	P (%)	K (%)	Na (%)	Cl (%)	Mg (%)	Mn (mg/kg)	Zn (mg/kg)
Control	1.744B**	0.304C**	0.535AB*	0.013B**	0.154B**	0.148C*	77A**	20B**
4 dS/m	1.84AB**	0.35BC**	0.515AB*	0.015B**	0.159B**	0.158B*	65A**	25A**
6 dS/m	1.89AB**	0.366B**	0.473B*	0.027B**	0.213B**	0.142D*	44B**	25A**
8 dS/m	2.167A**	0.455A**	0.577A*	0.123A**	0.393A**	0.171A*	44B**	27A**

With due attention to table 4 by increasing of salinity levels amount of nitrogen, phosphorus, sodium, chlorite, and zinc at grain increased, but manganese decreased. Further, maximum amount of potassium obtained from 8dS/m, and minimum amount of K obtained from 6dS/m treatment.

Maximum amount of Na and Cl obtained from 8dS/m treatment, that high significantly different from other salinity treatments. Also maximum Mg obtained from 8dS/m treatment, but different between treatments are at 5 percent. Trend of variation of Mn and zn is completely inversed. By increasing of salinity amount of Mn decreased and the least amount of it's obtained from 8dS/m treatment. But Zn of grain increased with increasing of salinity levels, and its maximum amount obtained from no salinity water. Maximum amount of Fe obtained from G28 at 4dS/m treatment.



**Figure2. Mean amounts of nutrient elements at grain of different varieties.**

Maximum amount of nitrogen at grain observant in Hassani cultivar, that it's high significantly different from other cultivar. Also the most amount of Phosphorous was accumulated in Hassani grain. Maximum amount of chlorite accumulated in Rahmatabadi cultivar that it cultivated in brackish Corbal Area. The most amount of Magnesium accumulated in Hassani cultivar, and after it arrangement Rahmatabadi, and the less amount of this element there is in G28, and Shahpasand Cultivars.

### Conclusion

Vast area in this region affected from salinity that cultivated paddy. There is lacking of suitable salinity tolerant rice Variety for south of Iran. Therefore, new research to find opportunity salinity resistant varieties with high quality and yield is necessary. In this research between 4 varieties G28 was better than others, because it is a native variety and agreeable for this region. The most sensitive varieties to salinity at this region was Hassani, and had the least yield and yield components. Shahpasand varieties had good vegetative growth, but at pollination stage was very sensitive to salinity, it can not to fill its grain. Quality and nutrient elements of rice grain affected from salinity, that between varieties G28 was the best and Hassani was the worst. Finally by increasing of salinity levels decreased quantity and quality of rice.

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