MANAGING C AND N FOR SUSTAINING SOIL QUALITY AND ENHANCING PRODUCTIVITY OF RICE IN RICE-RICE AND RICE-WHEAT SYSTEMS

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水稲連作および稲－小麦輪作体系における水稲生産性増進と土壌性保持のための炭素・窒素管理

有機・無機肥料の複合利用が生物的窒素固定を促進し、土壌肥沃度や土壌質・水稲生産性の維持･改善にどう影響するかを研究した。水田における窒素固定性藻類は1作あたり 13-25 kg N ha^{-1} 窒素固定できるのに対して稲と共同した従属栄養性窒素固定は約 1-5 kg N ha^{-1} と見積もられた。藻類に固定された窒素の 27-36%は 1～2 作目に有効になった。さらに 2 作後、重窒素標識した藻類由来窒素の 57%は土壌中に残存しており、無機肥料由来窒素で 30-40%であったのに比べ損失が少ないことが示された。ワラ施用は稲と共同した従属栄養性窒素固定を促進し、さらに植物あたりの窒素固定量は有機・無機肥料の施用によって影響を受けないか促進され、その効果は植物バイオマス量に比例した。圃場試験で窒素固定を促進し窒素利用効率を改善する品種が見出された。そうした品種の育成と利用は窒素投入要求を低減化するのに有効であろう。

アジアにおける長期稲連作と稲－麦輪作試験の解析結果から、無機肥料だけでは収量低下傾向が見られるのに対して堆厩肥を併用すると増収傾向が得られることが認められた。しかし初期収量は堆厩肥のみでは無堆厩肥より低く、試験 15 年目以降から逆転することがわかった。福岡（40 年間）、Ludhiana（インド、20 年間）および Bhairahawa（ネパール、15 年間）の 3 箇所から採取した堆厩肥連用と対照土壌の理化学性および微生物性の分析結果から、有機物、特に稲ワラ堆肥と厩肥は土壌中の全炭素・全窒素含量を最も増大させ、無施用に較べて土壌理化学性と微生物性を改善することが明らかになった。Ludhiana では全炭素量は増加または安定傾向を示したが、厩肥区以外では可分解性炭素と全窒素量は減少した。これらの結果から、無機肥料のみでは土壌肥沃度と生産性を維持できないことが明らかになり、最適な持続可能な有機物施用戦略の開発が必要であるといえる。

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SYNOPSIS

The impacts of the combined use of inorganic and organic fertilizers with enhanced biological nitrogen fixation (BNF), in improving or maintaining soil fertility, soil quality and productivity of rice were studied. The N contribution of an N_2-fixing algal bloom in the rice field was estimated at 13-25 kg N ha^{-1} while heterotrophic and rice plant-associated BNF was estimated at 1-5 kg N ha^{-1} $crop^{-1}$. It was shown by pot and field experiments that 27 to 36% of BGA-^{15}N was available to the first and second crops. Moreover, after 2 crops, 57% of ^{15}N from BGA as compared to 30-40% of ^{15}N from ammonium sulfate remained in the soil suggesting that algal N is less susceptible to losses than mineral fertilizer. Straw application supported heterotrophic and plant-associated BNF. Moreover, BNF per plant was either enhanced or not affected by the application of inorganic N and organic manures and the effect was proportional to the plant biomass. Rice varieties with higher ability to stimulate BNF and improved N utilization efficiency were observed in field screening trials. The development and preferential planting of such varieties would help reduce N input requirements.

An integrated analysis of rice yield data from rice-rice (RR) and rice-wheat (RW) long-term experiments (LTE) in Asia showed a declining trend with only inorganic fertilization and a positive trend when combined with farmyard manure (FYM). However, the initial yield was generally lower with FYM than without, that a yield increase due to FYM was observed only after 15 years. Soils from 3 RW LTE in Fukuoka, Japan (40y), Ludhiana, India (20y), and Bhairahawa, Nepal (15y), with continuous applications of organic manure (rice straw, wheat straw, rice straw compost, green manure, or FYM) combined with inorganic fertilizers (NPK) were analyzed for changes in soil chemical and microbiological parameters relative to unfertilized and inorganically fertilized controls. Addition of organic manures especially rice straw compost and FYM, which had the most accumulation of total C and N in the soil, showed improvements in soil chemical and microbiological properties over the controls. In Ludhiana, total C was increasing or was stable over the years but decomposable C and total N were decreasing except in the FYM treatment. Results indicate that inorganic fertilization alone cannot maintain soil quality and sustain productivity. Development of optimized but sustainable organic-supplemented fertilization strategies is needed.

GENERAL ABSTRACT

It has long been recognized that the management of soil organic matter (SOM) is of great importance for crop production and soil sustainability. In intensively irrigated lowland rice-based systems, research on SOM management has addressed short-term yield responses to the principal types of organic amendments: crop residues, green manures and animal manures. N_2-fixing blue-green algae (BGA), that is usually as rich in nutrients, or richer than many green manures is another source of organic manure contributing to paddy soil fertility. However, less is known about the long-term effects of organic manures on soil quality and crop productivity. This study was conducted to assess the short- and long-term (cumulative) contributions of various organic manures in combination with inorganic fertilizers to soil sustainability and rice productivity in rice-rice (RR) and rice-wheat (RW) systems.

The chemical composition of several species of BGA grown in the laboratory and greenhouse was determined. Using the average N values of 5 and 2.5% obtained from laboratory and field samples respectively, the potential contribution of a N_2-fixing algal bloom was calculated as 13-25 kg N ha^{-1} assuming a maximum blue-green algal biomass of 500 kg dry weight ha^{-1}. From the ^{15}N pot and field experiments, it was found that availability of ^{15}N from BGA ranged between 23 and 28% for the first crop and between 27 and 36% for the first and 2nd crops. It was also found that after two crops, 57% of ^{15}N from BGA as compared to 30-40% of ^{15}N from ammonium sulfate remained in the soil suggesting that algal N is less susceptible to losses than mineral fertilizer.

In addition to BGA, rhizospheric N_2-fixing bacteria also contribute to the nitrogen economy of flooded soils. More N is fixed in the presence of rice plants than in their absence, and at heading stage differences among varieties were most evident as measured by acetylene reduction. Activities ranged from 0.4 to 2 μmol C_2H_2 $plant^{-1}$ h^{-1} or 1-5 kg N ha^{-1} $crop^{-1}$. Straw application enhanced heterotrophic and plant-associated N_2-fixation and increased the population of total and N_2-fixing heterotrophs in the soil. Higher bacterial population was observed with surface application of straw as compared with incorporated straw.

Although it is possible to obtain moderate yields without any N input due to biological nitrogen-fixation (BNF), it is more often necessary to add inorganic and/or organic N inputs to increase yields. Besides, urea, grain legume green manure (*Vigna radiata* and *Dolichos lablab*), non-grain legume green manure (*Sesbania* and *Crotoloria*),

non-legume green manure (*Azolla*), and rice straw were also used as N sources. Nitrogen fixation per plant (or per unit area) was either enhanced or not affected by the application of inorganic N and organic fertilizers. However, when expressed on a per unit plant dry weight basis, the ARA was generally lower except in cases where some legume green manures such as *V. radiata* and *Crotolaria* were incorporated.

For the efficient management of soil C and N, the development and preferential planting of varieties that have higher N uptake and/or utilization efficiency are essential, as it will result in lower N input requirements. Field screening trials were conducted in two dry seasons to assess the variability among 180 rice genotypes of different growth durations in grain yield, N uptake and N utilization, without the addition of N fertilizer and to identify genotypes with the potential to produce high yields at suboptimal N levels through efficient uptake and/or utilization of N. Genotypes varied in their response to change in available soil N. The average increase in grain yield for each kilogram increase in N uptake was 61.9 - 82.7 kg. However, some genotypes absorbed similar amounts of N but produced different grain yields and/or total dry matter. The relative performance of genotypes in terms of NUE was more consistent than plant N uptake, based on rank correlations between the two trials. High N uptake and NUE were observed in IR131429-150-3-2-1-2 (NUE 65.4, N uptake 9.1 g m^{-2}) in the early-duration group, IR44 (NUE 67.2, N uptake 8.3 g m-2) in the medium duration group, and IR39323-182-2-3-3-2 (NUE 64.8, N uptake 9.3 g m-2) in the late-duration group. The study identified genotypes which may possess promising traits for improved N uptake and utilization efficiency.

Long-term experiments (LTE) provide the only direct method to determine the sustainability of fertilizer management systems. Data from LTE which began in the 1970s and 1980s, in RR and RW systems in Asia were used to analyze yield trends. Integrated analyses using the random regression coefficient- (RRCA) and meta-analysis showed a declining rice yield trend with only inorganic fertilization and a positive trend when combined with farmyard manure (FYM). However, the initial yield was generally lower with FYM than without, that a yield increase due to FYM was observed only after 15 years.

Soils from 3 RW LTE in Fukuoka, Japan, Ludhiana, India, and Bhairahawa, Nepal, with continuous applications of an organic manure combined with inorganic fertilizers (NPK) were analyzed for changes in soil chemical and microbiological parameters relative to unfertilized and inorganically fertilized controls. In Fukuoka, all the organic residue treatments (rice straw, rice straw compost, and wheat straw) with or without N

brought about significant increases in total C and N, permanganate-oxidizable C (MnOC), hot-water-extractable C (HWEC) and potential mineralizable N (PMN) as compared with the controls after 40 years. The highest accumulation of total C (23%) and N (72%) in the soil was from rice straw compost as compared with that from rice straw (C, 7% and N, 33%) and rye grass / wheat straw (C, 9% and N, 29%). Incorporation of rice straw compost also increased MBC under both aerobic and flooded conditions. An efficient utilization of C by microorganisms was indicated by a significantly lower metabolic quotient (qCO_2) in the composted and uncomposted rice straw treatments as compared with that in the unfertilized treatment under aerobic condition.

In Ludhiana, total pools of C and N, and HWEC were 28-40% higher than the control after 20 years only in the FYM+NPK plots where HWEC was also maintained with time. HWEC showed a declining trend over the years in the other treatments (100% NPK, wheat straw(WS)+NPK and green manure(GM) +NPK) while total C increased by 17% on the average from the 5^{th} to the 20^{th} year. In Bhairahawa, though total organic C and N increased with an organic amendment, HWEC did not. Accumulated percent increases in C and N as fractions of the applied organic fertilizers were 11-23 and 37-39 from FYM, 4-21 and 19-41 from GM, and 3 and 24 from WS respectively. Farmyard manure also improved available P, cation-exchange capacity (CEC), potential mineralizable N (PMN) and dehyrogenase activity, but microbial biomass C (MBC), basal respiration, flush of CO_2 after rewetting dried soil, and the metabolic quotient (qCO_2) remained unchanged.

Results from this study demonstrate that the current practice of inorganic fertilization alone can not maintain soil quality needed to sustain current levels of crop productivity. However, organic manures especially composted rice straw and FYM combined with inorganic fertilizers showed improvements in productivity and soil quality in the long-term. Available organic resources must be utilized for the development of optimized but sustainable organic-supplemented fertilization strategies to enhance rice productivity and soil quality.

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CHAPTER 1
GENERAL INTRODUCTION AND OBJECTIVES

1.1 General Introduction

1.1.1 C and N dynamics in wetland soils

The retention of floodwater in wetlands results in decreased rate of decomposition and consequently, increased accumulation of organic matter. Because of their relatively high primary production and low decomposition rate, relative to upland ecosystems, wetlands are considered net sinks for organic C and N (Debusk et al., 2001).

1.1.1.1 Carbon cycling in wetlands

In most soils, C is organic in nature and constitutes approximately 50% of soil organic matter. It serves a structural and a functional role in soil and as the driving force for transformation processes. At the agroecosystem or field scale, organic matter influences many readily measurable soil functions or processes (Schnitzer, 1991). Organic matter is both a source and a sink for plant nutrients, and provides an energy substrate for soil organisms. Soil macro- and microaggregation, that aid the infiltration of air and water, are promoted and stabilized by soil organic matter (Tisdall, 1996). Organic matter promotes water retention and influences the efficacy and fate of applied pesticides (Gregorich et al., 1994,1997). It also influences certain soil physical processes such as compactibility (Soane, 1990), friability (Watts and Dexter, 1998) and the range of soil available water for plant growth (Kay, 1998).

Organic C undergoes complex cycling in wetlands, and the fate of C depends on the specific type of compound, as well as environmental conditions. In general, easily degradable (labile) fractions are decomposed to inorganic constituents, while recalcitrant pools are accreted as new peat layers. Organic matter associated with above- and below-ground plant, algal, and microbial biomass in wetlands consists of complex mixtures of non-humic substances, including particulate pools (cellulose, hemicellulose, tannins and lignins, proteins), water-soluble components (amino acids, sugars, and nucleotide bases), and ether-extractable components (lipids, waxes, oils). As plants and microorganisms senesce and die, non-humic substances are deposited in the water column and soil surface. Labile fractions undergo multistep conversion to inorganic constituents including (1) abiotic leaching of

water-soluble components and fragmentation of tissues into small pieces (<1mm), (2) extracellular enzyme hydrolysis of biopolymers (e.g. nucleic acids, proteins, and cellulose) into monomers, and of biopolymers (e.g. nucleic acids, proteins, and cellulose) into monomers, and (3) aerobic and anaerobic catabolism of monomers by heterotrophic microorganisms. Microbial biomass has been found to make up a significant amount of C in wetland substrates. Nutrients that are released are also available for plant uptake and growth.

Extracellular enzymes such as cellulases and proteases are excreted by fungi and bacteria, and hydrolyze high-molecular-weight biopolymers into oligomers and monomers that can be taken up by microorganisms. Enzyme hydrolysis is generally considered the rate-limiting step in organic matter decomposition (Sinsabaugh et al., 1993). Oxidoreductase enzymes, such as peroxidases and phenoloxidases are involved in lignin oxidation. Because these enzymes require O_2, degradation of lignin is most prevalent in aerobic zones such as soil/water and root/soil interfaces. Production and activity of extracellular enzymes is affected by a number of environmental factors, including nutrients, pH, O_2 supply, humic substances, and inorganic cations.

Detrital material derived from roots and above-ground plant and algal biomass that are buried undergo anaerobic decomposition. Under anaerobic conditions, microorganisms must utilize alternate electron acceptors to oxidize organic matter. Due to energy differences, electron acceptors are utilized in the order $O_2 > NO_3^- > Mn^{4+} > Fe^{3+} > SO_4^- > HCO_3^-$. This phenomenon explains the stratification in microbial activity and chemicals often observed in wetland soil profiles (Reddy and D'Angelo, 1994). Slow decomposition rates under anaerobic conditions, partially explains why wetlands accumulate organic matter more than upland systems (DeBusk and Reddy, 1998).

Carbon compounds that are recalcitrant to aerobic and anaerobic decomposition tend to accumulate in wetlands, either as undecomposed plant tissues (peat) or humic substances. Formation of humic substances is thought to involve condensation reactions between reactive phenolic groups of tannins and lignins with water-soluble non-humic substances, catalyzed by phenoloxidase enzymes in soils (Stevenson, 1994). This mechanism accounts for high molecular weight, and heterogenous humic substances that contain significant amounts of N, P, and S in their structures. In the absence of O_2, humic substances are resistant to

decomposition, and thus represent a significant carbon and nutrient storage in wetlands. Under drained conditions, humic substances are rapidly degraded, which releases nutrients to the bio-available pool, thereby affecting downstream water quality.

1.1.1.2 N cycling in wetlands

Nitrogen exists in a variety of inorganic and organic forms in wetland soils, with the majority present in organic forms. The various forms of nitrogen in the soil include 1) soluble mineral forms: ammonium, nitrate, nitrous oxide (gas), 2) soluble organic compounds e.g. urea, amino acids, 3) living organisms: plant roots, fungi, bacteria, soil animals, 4) insoluble forms: SOM (decomposable dead organisms, cell debris, humus), ammonia bonded to clays (Bockman et al., 1990).

Organic N stored within wetland soils is the most stable pool of N and, as such, is not readily available for internal cycling. As with C, the more refractory organic compounds become buried within the soil and slowly accrete over time. There are a variety of factors that control the stability of the accreted organic N in the soil, including water depth, hydrologic fluctuations, temperature, supply of electron acceptors, and microbial activity.

In flooded soils, the inorganic N is present mostly as NH_4^+ with only trace amounts of NO_3^- and NO_2^-. Gaseous N products include NH_3, N_2O, and N_2 and are generally transient stores as they are released to the atmosphere. The inorganic stores of N are not very stable over time and generally comprise less than 1% of the total N within the wetland (Howard-Williams and Downes, 1994).

The microbial biomass N compartment is relatively small when compared with the total pool of organic N, yet the microbial activity is the single greatest regulator of the stability of organic N. Microbial decomposers utilize simple organic N compounds for cell growth, while a variety of functional microbial groups utilize inorganic N compounds as key components in the electron transport phosphorylation system. The percent of microbial biomass N with respect to total N is typically within the range of 0.5 to 3% of total N (White and Reddy, 2000).

The microbial conversion of nitrogen in organic residues and soil organic matter into soluble forms is called mineralization. In this process, carbon compounds are degraded and used as a source of energy. Nitrogen in excess of the microbial need is

liberated. Soluble nitrogen forms are also liberated through the death and rupture of cells.

The result of the mineralization of plant residues depends (among other factors) on the carbon/nitrogen ratio of the residues. As long as this is high, more than about 30:1, nitrogen will be bound in the microbes that decompose the material; if the ratio is below 20:1, nitrogen liberation starts early in the decomposition. Depending on the crop, the amount of residues and the carbon nitrogen ratio, the nitrogen set free from the previous year's residues can range from 0 to some 150 kg per ha (Bøckman et al., 1990).

Inorganic N in wetlands undergo the processes of nitrification and denitrification, ammonia volatilization, and plant uptake. Ammonification is the primary step in organic N mineralization and is defined as the biological process by which organic forms of N are transformed to NH_4^+. Similar to organic C, complex polymeric organic N compounds are hydrolyzed to simple monomers through the activity of extracellular enzymes (Gardner et al., 1989; McLatchey and Reddy, 1998). Eventually, this process leads to the final breakdown of amino acids and results in the liberation of NH^{4+}.

Ammonification is carried out by a wide variety of general-purpose heterotrophic microorganisms and occurs under both aerobic and anaerobic conditions. Ammonium concentrations increase under anaerobic conditions as a result of the low N requirements of anaerobic microorganisms. This increase is also due to the absence of oxygen, which prevents nitrification, the transformation of NH_4^+ to NO_3^-. Immobilization is the conversion of inorganic N to organic forms. The difference between immobilization and ammonification is termed "net N mineralization" and is generally a positive value in wetlands, again due to the lower N requirements of the anaerobic microbial community.

The soil microbial biomass helps regulate the transformation and storage of nutrients in soils (Martens, 1995). The size and activity of the microbial pool have been shown to significantly correlate with net N mineralization rates in wetland soils (Williams and Sparling, 1988; White and Reddy, 2000). Ammonification rates have been reported to range from 0.004 to 0.357 g N m^{-2} d^{-1} in wetland soils (Martin and Reddy, 1997). Ammonium N released through the mineralization process is readily available to plants and the microbial pool. Depending on the environmental

conditions, NH_4^+ can either be lost through volatilization and/or oxidized to nitrate N.

Ammonia volatilization is an abiotic process controlled by the pH of the soil-water system. The rate of volatilization increases dramatically over the pH range from 8.5 to 10. Losses due to volatilization are insignificant at a pH < 7.5.

Nitrification is an obligate aerobic process involving oxidation of NH_4^+ to NO_3^-. It is mediated by autotrophic bacteria that couple the oxidation of NH_4^+ to electron transport phosphorylation while utilizing inorganic C to synthesize required cellular components. The process of nitrification is a two-step process that first involves the oxidation of NH_4^+ + to NO_2^- by bacteria of the genus *Nitrosomonas*. The final step, the oxidation of NO_2^- to NO_3^- is mediated by bacteria of the genus *Nitrobacter*. Nitrification occurs in three zones of a wetland soil-water profile: water column, aerobic soil layer, and the aerobic region of the rhizosphere. In all of these zones, the supply of ammonium and the availability of oxygen are the major limiting factors for nitrification.

Nitrate added to wetlands or derived through nitrification, rapidly diffuses into anaerobic soil layers where it is utilized as an electron acceptor and reduced to gaseous end products of N_2O and N_2 or NH_4-N. The former pathway known as denitrification occurs at higher Eh levels (200 to 300 mV) while the latter process known as dissimilatory NO_3 reduction to NH_4^+, occurs at low Eh level (<0 mV).

Denitrification is the microbial mediated reduction of nitrogenous oxides to N_2 gas. Denitrifiers are known to exist in almost all soils and come from a wide range of genera, including Pseudomonas, alcaligenes, Flavobacterium, Paracoccus, and Bacillus (Firestone, 1982; Tiedje, 1988). The facultative anaerobic bacteria are capable of using NO_3^- or NO_2^- as terminal electron acceptors, coupled with the oxidation of organic C in the absence of O_2.

In general, the relatively high organic C in wetland soils provides ample substrate for heterotrophic microbial activity. Due to the high C content and paucity of O_2 in wetland soils, NO_3^- supply becomes the limiting factor in dentrification (Cooper, 1990; Gale et al., 1993; White and Reddy, 1999). Denitrification rates in wetlands have been reported to range from 0.003 to 1.02g N m^{-2} d^{-1}. Denitrification is the primary mechanism for inorganic N removal from the wetland systems, and, as such, wetlands are utilized for their significant water quality enhancement capabilities.

Biological nitrogen fixation (BNF) is the only mechanism available for conversion of inert gaseous N_2, present in the atmosphere, to biologically available organic and inorganic forms. The process of N_2-fixation is driven by the nitrogenase enzyme produced by only a limited number of prokaryotic organisms including several genera of bacteria and cyano bacteria (Vymazal and Richardson, 1995).

There are 2 major sources of BNF in lowland rice systems: a) Indigenous BNF, arising from diazotrophs including heterotrophic and phototrophic bacteria and blue-green algae (BGA) or cyanobacteria, which are indigenous in the soil-rice-floodwater system and b) exogenous BNF, arising from diazotrophs such as phototrophic rhizobia associated with aquatic legumes (e.g. *Sesbania* and *Aeschynomene* species), which are exogenous to the soil-rice-floodwater system).

Aquatic conditions in rice fields provide an optimum environment for indigenous BNF by diverse autotrophs and heterotrophs. Submerged soils with BGA at the surface and N_2-fixing bacteria in the bulk of the soil are favorable environments for BNF. The submerged environment is suited to both aerobic and anaerobic N_2-fixing bacteria. Aerobic BGA can thrive in the oxygenated surface layer and diverse bacteria (aerobic, microaerobic, and anaerobic) occur in the soil and the rhizosphere of rice. The presence of rice plants creates an environment favorable for BNF because air, which is 78% N_2, is transported down the shoot to the root of rice where it diffuses out into the soil and in contact with N_2-fixing bacteria. This ensures a supply of N_2 for aerobic fixers in the rhizosphere. More N is fixed in the presence of rice plants than in their absence.

Biological N_2-fixation can occur in soil-rice-floodwater systems throughout rice growth and it can occur in rice soils between crops particularly when the soil remains saturated or submerged. Long-term N balance experiments in intensive irrigated rice systems have shown N contributions of about 15 to 50 kg N ha^{-1} $crop^{-1}$ from nonsymbiotic N_2- fixation.

Normally, one to two crops of rice is grown annually in humid tropics with a fallow period between two crops. Aquatic plants like the water fern (*Azolla*) grown in floodwater before and during the rice crop can be a source of N. Legumes (Sesbania or Aescynomene) raised during the fallow period as green manure crops can be used as a source of N for the following rice crop.

A wide range of biological N_2-fixation rates are reported for wetlands. Rates have been reported to range from 0.7 to 12 g N m^{-2} yr^{-1} in the root zone, and from

0.4 to 46 g N m^{-2} yr^{-1} within the photic zone of coastal wetlands (Buresh et al., 1980). The presence or absence of vegetation can affect the distribution of and N_2-fixation rates. Well-developed cyanobacterial mats have demonstrated rates ranging from 1.2 to 76 g N m^{-2} yr^{-1}, while soils devoid of macrophyte coverage or cyanobacterial mats have demonstrated rates in the range of 0.002 to 1.6 g N m^{-2} yr^{-1} (Howarth et al., 1988).

The overall cycling of organic N to inorganic N and through to removal from the wetland follows a path beginning with mineralization of organic matter to NH_4^+. Ammonium produced in the primarily anaerobic soil will be conserved until diffusion into an aerobic water column or soil volume. Nitrification constrained to these aerobic zones, is responsible for the transformation of NH_4^+ to NO_3^-. Nitrate is then conserved until diffusion into the anaerobic zones of the soil, where it becomes converted to either N_2O or N_2 gas, leading to removal from the wetland system. The time of cycling of N through the wetland is short in comparison with the residence time of N_2 gas in the atmosphere. However, the transformations of N from N_2 gas through the various bioavailable (inorganic) forms are critical in maintaining wetland ecosystem productivity.

1.1.2 Characteristics of soils under rice-wheat systems

Rice and wheat have been grown as food crops in Asia for more than 6000 years. The rice-wheat cropping system has been practiced for 1000 years (Tran and Marathee, 1994) but the intensification that exists today evolved rapidly since the 1960s after the introduction of modern high yielding varieties, access to irrigation, fertilizer, and better infrastructure. These systems provide staple grain supply for more than 400 million people, or about 8% of the world's population and are critical for the food security of Asia.

Contrasting edaphic requirements of rice and wheat grown in sequence on the same piece of land calls for special management practices. The dominant characteristic of the system is the repeated transition from the anaerobic conditions for rice to aerobic conditions for wheat. This has great impact on the soil physical properties, as well as on the chemical reactions of indigenous and exogenous nutrients in the soil (Timsina and Connor, 2001).

1.1.2.1 Physical characteristics

Puddling breaks capillary pores, reduces void ratio, destroys soil aggregates, disperses fine clay particles, and lowers soil strength in the puddled layer (Sharma and De Datta, 1986). It helps control weeds, improves water and nutrient availability, and facilitates transplanting. In continuous rice culture, soil compaction can be seen as an investment in infrastructure. But in the R-W system, the hard pan and the destruction of the structure of the surface soil are serious liabilities to the performance of wheat and other crops that require aerated soils. Puddling, retains water by compacting the subsoil and provides a readily accessible, but shallow, root zone for rice crop establishment. Repeated puddling restricts water loss by percolation (Greenland, 1985; Sharma and De Datta, 1986; Painuli et al., 1988; Humphreys et al., 1994). In such soils, rice roots are largely confined within the puddle layer and a few penetrate below 20 cm. This results from the shallow-rooting behavior of rice and the physical resistance to penetration offered by the compacted layer (Beyrouty et al., 1987).

The anaerobic condition of flooded soils immediately after rice cropping, the impaired soil structure of the puddled layer and the compacted layer that strengthens to form a hard pan of increased strength on drying are major impediments to the establishment and growth of ensuing crops (Meelu et al., 1979; Prihar et al., 1985; Gajri et al., 1992, 1999; Aggarwal et al., 1995; Kirchhof and So, 1996). The deleterious effect depends upon soil type. The destruction of soil aggregates by puddling leads to the formation of surface crusts and cracks on drying, delaying preparation of a seedbed for ensuing crops. When broken by tillage, the resulting large clods provide poor contact with seed, thereby restricting germination. If crusts form following rain soon after sowing, late-emerging seedlings may be trapped below the surface.

Subsurface compaction caused by puddling generally reduces root growth of wheat (Oussible et al., 1992; Aggarwal et al., 1995), although roots that penetrate the compacted layer before it hardens on drying may extend more deeply as the soil drains (Sur et al., 1981). Little is known of root growth of wheat in R-W systems on puddle soils of heavy texture.

Puddling of soils for rice destroys soil macro-aggregates leading to substantial losses of SOC in the rice-wheat system. An example for a soil initially high in SOC is the mollisol at Pantnagar, where 20 years of cropping reduced SOC from 1.48% to 0.49% and 0.84% in unfertilized and recommended NPK treatments, respectively

(Ram, 2000). High losses of SOC in the rice-wheat system contrast with results from double and triple cropped long-term rice experiments where SOC levels are maintained or even increased due to differences in decomposition patterns and products under more continuous maintenance of anaerobic conditions.

1.1.2.2 Chemical characterisitics

Flooding of rice fields causes major chemical changes in the soil that affect the transformation and availability of nutrients, carbon dynamics, and growth of rice and subsequent crops (Ponnamperuma, 1972, 1985). On flooding, a molecular oxygen is depleted, and NO_3^-, Fe(III), Mn(IV), and $SO4^{2-}$ are chemically reduced. These transformations increase the availability of P, K, Si, Mo, Cu, and Co, and decrease those of N, S, and Zn (Patrick, 1982; De Datta and Patrick, 1986; Sanyal and De Datta, 1991). Anaerobic conditions also promote the production of methane and toxic products such as organic acids and hydrogen sulfide by microorganisms as they decompose organic substrates (Ponamperuma, 1972).

Flooded soils differ from others in the control of acidity and alkalinity because the partial pressure of CO_2 in floodwater buffers carbonate and lowers pH. Soils that are initially acid when flooded tend to equilibrate with the floodwater and become less acidic within a few days, while those that are initially alkaline and sodic move towards neutrality, but more slowly. These pH changes alter chemical equilibria and consequently the availability of nutrients.

Most chemical changes are reversible on draining giving important implications for nutrient management in R-W systems. For sustained high productivity, the major concern is for the effect of flooding and draining on the availability of macronutrient, N, P, and K.

1.1.2.3 Biological characteristics

Biological activity in soil is primarily determined by the supply of easily degradable organic matter. When an adequate supply is available, nitrogen may limit microbial activity for short periods. In a field situation, the major determinant of microbial soil activity is its content of organic carbon. This in turn is related to the crop rotation used and the proportion of the crop returned to the soil.

Many studies have focused on the influence of drying and rewetting on microbial activity and decomposition of SOM (Soulides and Allison, 1961; Bloem et al., 1992;

Magid et al., 1999). Dry-wet cycles generally cause an increase in decomposition of SOM, because more decomposable organic substrates become available for microbial attack upon drying and rewetting (Soulides and Allison, 1961; Sorensen, 1974). These substrates are derived partially from the death of a portion of soil biota upon drying (Lund and Goksoyr, 1980; Bottner, 1985) and partially from the release of previously inaccessible organic compounds occluded in aggregates (Sorensen, 1974; Van Gestel et al., 1991). The increased availability of organic substrates results in a flush of microbial activity and an increase in length of fungal hyphae and bacterial biomass (Jager and Bruins, 1974).

Few organisms are adapted to both aerobic and anaerobic conditions and the repeated transition between them limits the survival of disease-causing organisms in R-W systems. Weeds, pests, and disease problems are most likely to be caused by organisms that are mobile, or are readily reintroduced by wind, water or other organisms. These considerations of survival also relate to beneficial organisms, the most important of which are the microorganisms responsible for the fixation of atmospheric N and its transformations in the soil.

1.2 General Objectives

The impacts of agronomic and soil management practices involving the combined use of inorganic and organic fertilizers and biological nitrogen fixation (BNF) on improving or maintaining soil fertility, soil quality and productivity were studied. The general objectives of this study were:

a. To assess the contribution of nitrogen-fixing blue-green algae and bacteria to paddy soil fertility.
b. To determine the effects of inorganic and organic fertilizer management on BNF
c. To assess rice genotypic variability in ability to stimulate BNF, N uptake and N use efficiency
d. To determine long-term effects of inorganic and organic manures on yield trends in rice-rice and rice wheat systems
e. To assess various chemical and biological soil parameters for measuring SOM quality and quantity in rice-rice and rice-wheat systems.
f. To determine long-term effects of inorganic and organic manures on some soil quality parameters.

g. Determine the interrelationships among various chemical and microbiological soil quality parameters

CHAPTER 2

CONTRIBUTION OF BLUE-GREEN ALGAE AND RICE PLANT-ASSOCIATED NITROGEN FIXERS TO THE SUSTAINABILITY OF PADDY SOILS

2.1 Summary

It has been established that biological nitrogen fixation (BNF) by blue-green algae (BGA) and heterotrophic bacteria plays a vital role in the maintenance and buildup of paddy soil fertility. However it is important to know how much, when and how the fixed nitrogen is made available to the rice plant.

Assuming a maximum blue-green algal biomass of 500 kg dry weight ha^{-1} and using average N values obtained from laboratory and field samples (5 and 2.5% respectively), it appears that the potential contribution of a N_2-fixing algal bloom is 15-25 kg N ha^{-1}.

The fate of nitrogen from blue green algae (BGA) was studied in pot and field experiments. Availability of ^{15}N from BGA incorporated into the soil ranged between 23 and 28% for the first crop and between 27 and 36% for the first and second crops. Surface application of the algal material reduced ^{15}N availability to 14-23% for the first crop and 21-27% for the first and second crops. The pot experiment demonstrated that for the first crop, algal ^{15}N was less available than ammonium sulfate, but for two crops, its availability was very similar to that of ammonium sulfate. After two crops, 57% of ^{15}N from BGA and 30-40% of ^{15}N from ammonium sulfate remained in the soil.

Plant-associated BNF as measured by acetylene-reducing activity (ARA) differed among rice varieties. It was at heading stage that differences among varieties were most evident and sampling variance was lowest. Activities ranged from 0.4 to 2 μmol C_2H_2 $plant^{-1}$ h^{-1}. Assuming that ARA measured at heading lasts 50 days, a C_2H_2:N_2 ratio of four, and a plant density of 25 m^{-2}, N_2-fixing rate would be 1-5 kg N ha^{-1} $crop^{-1}$. ARA of different varieties at heading was also positively correlated with total dry matter yield at maturity. Among the plant characters, root biomass appeared to be the most important factor affecting plant ARA. IR42 and IR50 consistently showed the highest ARA per plant or unit plant dry weight among the long and short duration genotypes in both seasons.

2.2 Introduction

The transfer of algal nitrogen to higher plants other than rice has been demonstrated qualitatively in natural ecosystems (Mayland and McInstosh, 1966; Stewart, 1970; Jones

and Wilson, 1989) using ^{15}N tracer techniques. Tracer experiments aimed at determining the availability of algal nitrogen to wetland rice and its fate in paddy soils have been mostly qualitative (Renaut et al., 1975; Venkataraman, 1977). Previous to this study, the only quantitative data were those from Wilson et al., 1980 who recovered from a rice crop 37% of the nitrogen from ^{15}N-labeled *Aulosira* sp. spread on the soil, and 51% of the nitrogen from the same material incorporated into the soil. Their study was conducted on a laboratory scale and did not include analysis of ^{15}N remaining in the soil.

Nitrogen fixed by free-living microorganisms in a wetland rice ecosystem normally enters the soil N pool after the microorganisms die. Rice plant-associated N_2-fixation on the other hand, has some advantages over other N_2-fixing systems: 1) a part of the fixed N is available to the plant immediately (Ito et al., 1980; Eskew et al., 1981; Yoshida and Yoneyama, 1980; Watanabe and Roger, 1984), and 2) plant-associated N_2-fixation is less sensitive to N fertilizer (Watanabe et al., 1981; Ladha, 1986). Furthermore, as most of the plant-associated fixed N is microbially bound in the rhizosphere, it is probably not readily amenable to loss processes unlike N fixed by photoautotrophs in the floodwater.

Plant plays an important role in supporting bacterial associative N_2-fixation by supplying some of its photosynthates to the submerged part of the plant. To enhance the contribution of biologically-fixed N in the rice plant, the plant's ability to support N_2-fixation and efficient utilization of the fixed N are important. Significant differences in the capacity of several rice genotypes to support N_2-fixation were reported (Lee et al., 1977; Hirota et al., 1978; Balandreau et al., 1978; Balandreau, 1980; Habte and Alexander, 1980; Sano et al., 1980; Barraquio et al., 1986; Ladha et al., 1986) and the need for simple and easy selection criteria has been emphasized (Ladha et al., 1986).

In this study the role of phototrophic, and rice plant-associated N_2-fixation in enhancing the sustainability of rice paddy fields were investigated in relation to soil and crop management practices.

2.3 Objectives

The objectives of this study were:

a. To determine the N contribution of N_2-fixing BGA in paddy soils and the availability of blue-green algal N to the rice plant using the ^{15}N tracer technique.
b. To compare the availability of N from surface-applied and incorporated BGA with that from surface-applied and incorporated ammonium sulfate under field conditions.

c. To detect variations among rice varieties in their N_2-fixation capability and stability

d. To determine variability in rice plant-associated ARA in relation to plant characters and environmental factors

2.4 Materials and Methods

2.4.1 Determination of the N contribution of N_2-fixing BGA in paddy soils and its availability to the rice plant using the ^{15}N tracer technique

2.4.1.1 Analyses of flask cultures and rice field samples of N_2-fixing BGA isolated from rice fields

Dry weight and ash were determined on pellets from algal suspension centrifuged at 10,000 rpm for 15 min. Dry weight was measured after 24 h of heating at 80°C in an oven, and ash content by heating the material at 325°C until smoking ceased and then 480°C overnight. Mineral contents were measured using analytical methods for plants of the IRRI analytical laboratory.

Carbon was analyzed by the Walkley and Black method (Black, 1965) using algal suspensions sonicated for 5 min in an ice bath. Nitrogen was measured by the standard micro-Kjeldahl method.

2.4.1.2 Preparation of ^{15}N-labeled Nostoc cells.

A unialgal *Nostoc* strain, isolated from a paddy field in Sri Lanka, was grown in 20-liter carboy bottles under continuous fluorescent light. The culture medium was a modification of the BG-11 medium (Allen and Stanier, 1968). The composition is as follows: (mg l^{-1}), $CaCl_2.2H_2O$, 36.0; K_2HPO_4, 30.5; NaEDTA,1.0; Mg $SO_4.7H_20$, 7.5; Fe^{3+}-NH_4-citrate, 6.0; Na_2CO_3, 100; H_3BO_3, 2.9; $MnCl_2.3H_2O$, 1.8; $ZnSO_4.7H_2O$, 0.2; $Na_2MoO_3.7H_2O$, 0.4; $CuSO_4.5H_2O$, 0.08; and $Co(NO_3)_2.6H_2O$, 0.05. The culture was continuously stirred with a magnetic stirrer and air-enriched with CO_2 was bubbled through it. The CO_2 concentration was adjusted to reach a pH of around 7 in the culture. The pH was indirectly measured by trapping outgoing air in a measuring cell filled with the same medium (Roger, 1974).

Every 2 or 3 days, algal cell density was determined by measuring the absorbance of phycocyanin (620-735 nm) and chlorophyll (680-735 nm) of an aliquot sonicated at 20 kHz for 4 min in an ice bath. When the algal density

reached 0.2 g dry weight l^{-1}, ^{15}N-labeled KNO_3 (75 atom% excess) was added at the rate of 0.14 g ^{15}N $bottle^{-1}$ (10.4ppm NO_3-N). Blue-green algae were grown until nitrate was consumed (10-12 days) and were harvested by decantation and moderate centrifugation (3000 rpm). The algal material was dried in an incubator at 40°C and was ground by pestle and mortar. Because the dessication was performed for several days at a temperature favorable for spore formation, the material was very rich in akinetes. Yields ranged from 10 to 12 g dry weight $bottle^{-1}$ for 12 days. The algal material from different batches was mixed thoroughly before use; it was composed of 1.13% P, 0.90% K, 0.74% Ca, 0.72% Mg, 38.9% C, 7.3% N, 5.28% C/N, and 23.0 atom % excess ^{15}N.

2.4.1.3 Pot experiment

Two kg (oven-dry weight) of puddled flooded soil from a field of the International Rice Research Institute (IRRI) farm (an Aquic Tropudalf) with pH 6.7, clay 56%, organic matter 2.24%, N 0.18%, was put into pots 18 cm in diameter. The pots were filled to a depth of 15 cm. The soil was allowed to stand for 2 days, and then the floodwater was drained off. Four treatments were established:

S-BGA: ^{15}N-labeled Nostoc sp. spread on the surface of the soil at a rate corresponding to 2 g N m^{-2} or 50.85 mg N pot^{-1} and 11.7 mg 15N pot^{-1}.

I-BGA: ^{15}N-labeled Nostoc sp. incorporated and mixed thoroughly through the total volume of soil at the same rate as S-BGA.

S-AS: ^{15}N-labeled ammonium sulfate (21.2% N, 28.55 atom% excess^{15}N, 14.53 mg ^{15}N pot^{-1}) spread on the soil surface at the same level of N as algal N.

I-AS: ^{15}N-labeled ammonium sulfate incorporated throughout the total volume of soil at the same rate as S-AS.

Nine replicates per treatment were used. After 1 day, the soil was submerged in about 3 cm water and 2 IR36 seedlings were transplanted per pot. Rice plants were cut just above the soil surface at panicle initiation (3 pots) and at maturity stage (6 pots). After the harvest of the first crop, soil and plants of three pots were analyzed for total N. Soils of the three pots were mixed and put into the pots again. The pots were kept flooded for 10 days before a second rice crop was

transplanted. After harvest of the second crop, plants and soils were analyzed. The upper 0-2 cm layer of the soil in the pots was scraped off and passed through a 2-mm sieve to separate weeds. Soil remaining in the pot was mixed thoroughly and passed through a 2-mm sieve to separate the roots. Total N and ^{15}N content of straw, grains, roots, weeds, and soils were determined. Total N was determined by the Kjeldahl method. ^{15}N was analyzed by emission spectrometry. The Dumas combustion method modified by Kumazawa (1974) was used for plants; the method of Yoneyama and Kumazawa (1974) for the soil.

2.4.1.4 Field Experiment

The experiment was conducted at the IRRI farm, on soil that had been continuously flooded and had not received nitrogen fertilizer for more than 5 years. The soil in each plot was mixed with a rotary weeder 3 times every other day. The soil was drained, and 2 metal square frames, 1.5 x 1.5 m and 40 cm in height, were placed to a depth of 15 cm from the soil surface.

Inside one frame, 65 g of labeled *Nostoc* corresponding to 2 g N m^{-2} or 1.245 g ^{15}N $plot^{-1}$, mixed with a small amount of air-dried soil, was spread by hand as evenly as possible on the soil surface. In the other frame, the same amount of ^{15}N-labeled Nostoc, mixed with a small amount of air-dried soil was incorporated by hand into the soil to a depth of 15 cm.

One day after the addition of algal material, the field was flooded. Three days after flooding, IR36 seedlings were transplanted inside and outside the metal frames at 20x20 cm spacing. Thirty-six rice hills (6x6 rows) were transplanted inside a frame.

After the first crop had been harvested, the field was flooded for 4 weeks, then a second crop was grown similarly. At maturity of both crops, the grain and the straw just above the soil surface were harvested. Three alternating rice plants from each row were combined to make up one composite sample per row. Thus, 6 composite samples were made from a frame. One day after harvest, soil samples were taken by inserting glass tubes 3 cm in diameter and 20 cm long into the soil up to a depth of 15 cm. A total of 10 composite samples (3 cores each) were taken from each frame, 5 from the center of 4 hills, and 5 near (5 cm away from) the rice hills. The upper 2-cm layer of soil was separated from the lower layer of the cores. Soil and plant N and their ^{15}N content were analyzed as in the pot experiment.

2.4.2 Measurement of plant-associated BNF (ARA) in 16 rice varieties over a growth cycle and related plant parameters

2.4.2.1 Field layout, treatments, and varieties

Field experiments were conducted in the dry (DS) and wet (WS) seasons at the IRRI farm (Aquic Tropudalf, pH 6.5, total N 0.14%, OM 2.9%, CEC 40 meq $100g^{-1}$, free iron oxide, 2.9%, available P, 14 ppm). The field which was an upland fallow for about 3 y was converted to lowland conditions about 1 month before the start of the experiment.

A randomized complete block design with three replications was used with 16 treatments (varieties). Each plot for treatment was 5 x 3.25m without bunds. The field was ploughed and harrowed 4 weeks before transplanting. A day before transplanting, superphosphate (Solophos, 20 kg P_2O_5 ha^{-1}) was applied and incorporated. Sixteen lines or varieties of *Oryza sativa* (Table 2.1), divided into short and long-duration [105 to115 and 125 to 140 days after transplanting (DAT) respectively] groups were used. Seedlings were raised for 20days on a wet seedbed, dipped in 2% ZnO solution, and transplanted in leveled plots at the rate of 4 seedlings per hill and spacing of 25 x 25 cm. The field was kept flooded throughout the experiment with a fallow period of about 3.5 months between the WS and DS crops. Pesticides were applied as needed for pest control.

2.4.2.2 Plant sampling and acetylene reduction (AR) assay

Two rice plants (hills) per block (total of 6 plants per variety) with almost similar tiller numbers (representative of the majority of plants in the plot) with rhizospheric soil were sampled from the second or third rows along the plant perimeter. Rice plants with a soil block were brought to the laboratory and AR assays of excised plant-soil system were performed (Barraquio et al., 1986). The assay involves digging the plant from the field, removing the surface soil and excess soil adhering to the roots, cutting the stem so as to leave about 5 cm of submerged portion of shoot, and finally placing the excised plant-soil system in a plastic bag. The plastic bag was then heat-sealed twice and air containing 25% acetylene (C_2H_2) plus a small quantity of (0.002%) of propane (C_3H_8) was introduced, and finally incubated at 35°C in the dark for 6 h. Ethylene evolved

was measured and calculated using propane as an internal standard. During the WS, AR assays of plants of the long-duration group were performed at 40, 55,

Table 2.1 Rice varieties and lines used for measurements of rice plant-associated N_2-fixation

Variety / Line	Growth duration (days)[a]
Long duration Group	
IR42	120-135
Rodjolele	120-140
IR20	10-125
Peta	120-140
IR13540	110-125
IR22082-4-1-2	115-130
IR18349-135-2-3-2-1	110-125
IR15314-30-3-1-3	115-130
Short duration group	
IR50	95-105
IR13240-82-2-3-2-3-1	100-115
IR13427-45-31-2-2-2(3107)	95-105
IR36	95-110
IR31802-48-2-2-2-3189	95-110
IR9729-67-3	90-105
IR21912-56-3-1-2-1-2	100-115
IR19728-9-3-2-3-3	90-105

[a] Ranges for the dry and wet seasons

74, 81, 88, and 94 days after transplanting, while plants of the short duration group were assayed at 40, 55, and 74 DAT. The last assays were made at heading of each variety. During the DS, plants of varieties belonging to both groups were sampled and assayed for ARA for three consecutive days only at heading stage. Six plants per variety (2 plants from each of the 3 blocks) per day (total of 18 plants per variety) were assayed. The mean ARA for each day and for 3 day measurements was determined.

2.4.2.3 Determination of plant dry weight and nitrogen

Dry weights of root and shoot of plants used for AR assays were determined. The reported root dry weights include the lower 7 cm submerged portion of the stem and leaf sheath. After AR assay, the roots were washed carefully to remove the soil. Plant samples were dried in the oven at 80°C for about 24 h and weighed. For the determination of harvest index, a 5-m^2 area per variety per block was harvested. Plants were cut from the ground and grains separated from straw, and the dry weight was obtained. For plant N determination, 16 plants per block per

variety were harvested and the N content was determined from ground samples of grains and straw from each block. The maximum content of vegetative N was determined from the plants harvested at heading stage and after separating developing grains, if any. Nitrogen content of the dried plant material was determined by the micro Kjeldahl method (Yoshida et al., 1976). Harvest index (HI) was calculated by dividing the weight of the grain by the weight of the total plant biomass above ground. Nitrogen harvest indiex (NHI) is equal to the weight of the N in the grain divided by the weight of total N in the plant biomass above ground. Nitrogen remobilization efficiency (NRE), a measure of the plant's ability to translocate N from the vegetative tissues to the developing grain, was calculated as follows (Creagan and Van Berkum, 1984):

NRE = (maximum vegetative N – final vegetative N)/maximum vegetative N

2.4.2.4 Trend of plant characters

The general trend of ARA and other plant characters (root, short dry weight, tiller number, and leaf area) over a growth cycle was obtained by analyzing six replicate plants per variety at 21 DAT and until about 20 days after heading at about 8 day intervals in 8 long- and 8 short-duration varieties.

2.5 Results

2.5.1 N contribution of N_2-fixing BGA in paddy soils and its availability to the rice plant

2.5.1.1 Analyses of N_2-fixing BGA isolated from rice fields and cultures grown in the laboratory

Analyses of 11 samples of field-grown BGA are presented in Table 2.2. Ash content was high and averaged 52%. Nitrogen content on an ash-free basis averaged 4.8%. Carbon content on an ash-free basis averaged 40% and exhibited low variability (CV=7%). The C:N ratio averaged 8.5 and ranged from 6.6 to 11.6. The phosphorus content averaged 0.1% and K 0.3%. Mg and Ca were high and exhibited large variability.

Average values and an analysis of the variability of C and N in BGA grown in flask cultures are presented in Table 2.3. Pooled data showed that dry matter averaged 3.72% and exhibited large variations (CV=66%). Lower values were

observed in the mucilaginous genera *Gloeotrichia* and *Nostoc*. Non-mucilaginous *Calothrix* and *Fischerella* have above average values.

Table 2.2. Analysis of field samples of N_2-fixing BGA (all data in percentage dry weight).

Sample	Ash	N	N ash free	C	C ash free	C:N	P	K	Mg	Ca
Aphanothece + *Nostoc* bloom	46.1	2.83	4.89	26.1	45.1	9.2	0.122	0.343	1.22	8.30
Nostoc commune	30.7	3.20	4.49	29.7	41.7	9.3	0.050	0.172	7.49	1.18
Mixed algal flakes	64.4	2.34	5.97	15.4	39.2	6.6	0.121	0.271	0.45	2.71
Aphanothece + *Nostoc* bloom	71.3	1.62	4.76	12.8	37.8	7.9	0.113	0.320	2.25	6.13
Aphanothece bloom	43.8	2.49	4.11	23.1	38.1	9.3	0.181	0.569	2.07	3.95
Nostoc bloom	55.9	2.76	5.63	18.9	38.7	6.9	0.159	0.475	1.56	2.10
Nostoc bloom	45.6	2.44	4.15	21.4	36.6	8.8	0.081	0.350	3.11	7.77
Aphanothece + *Gloeotrichia* bloom	58.8	1.75	3.82	20.4	44.5	11.6	0.074	0.388	3.29	7.20
Nostoc bloom	55.2	2.64	5.32	18.9	37.6	7.1	0.142	0.285	1.92	1.04
Aphanothece bloom	nd	2.93	nd	nd	nd	nd	nd	nd	nd	nd
Nostoc commune colonies	49.6	2.72	4.99	21.4	39.3	7.9	0.109	0.122	5.23	1.16
Mean	52.1	2.52	4.81	20.8	39.9	8.5	0.115	0.329	2.86	4.15
CV %	22	19	14	23	7	18	34	40	73	70
Maximum	71.3	3.20	5.97	29.7	45.1	11.6	0.181	0.569	7.49	8.30
Minimum	30.7	1.62	3.82	12.8	36.6	6.6	0.050	0.122	0.45	1.04

Table 2.3. Average generic values and variability of dry matter, C and N in N_2-fixing BGA grown in laboratory cultures.

Genera		Dry matter	N	C	C:N
Anabaena	n	6	6	5	5
	Mean	4.07	6.66	51.1	7.46
	CV	73	28	20	42
Calothrix	n	9	9	3	3
	Mean	6.26	5.38	35.6	6.61
	CV	13	20	10	24
Fischerella	n	4	4	3	3
	Mean	6.48	3.85	35.6	8.09
	CV	69	42	11	25
Gloeotrichia	n	6	6	2	2
	Mean	1.70	4.89	39.5	8.28
	CV	94	36	10	24
Nostoc	n	14	14	6	6
	Mean	2.62	4.83	42.2	9.12
	CV	61	26	10	34
Other genera	n	7	7	3	3
	Mean	3.81	4.10	nd	nd
	CV	Not taken into account			
Pooled data	n	46	46	22	22
	Mean	3.72	4.99	41.6	8.09
	CV	66	31	20	31
	Max.	13.1	8.26	67.3	13.2
	Min.	0.28	1.78	29.7	4.82

Nitrogen content averaged 5% and exhibited moderate variations (CV=31%) with values ranging from 3.40 to 8.26. Compared with other genera, Anabaena had a higher N content (6.7%). Carbon content averaged 41.6% and had the lowest variability (CV=20%). C:N ratio averaged 8.09 and ranged from 5 to 13. The two extreme values were observed in the same genus (*Anabaena*).

2.5.1.2 Availability of BGA-N to the rice plant (Pot experiment)

One week after transplanting, algal films developed on the surface of the floodwater in pots where *Nostoc* was spread on the soil surface. Microscopic examination revealed the presence of *Nostoc* filaments and spores, indicating that the applied dried BGA had recovered. The ^{15}N content of the algal films showed that ^{15}N was diluted from 23 atom % excess to 9%. No quantitative measurements were made to determine the contribution of the inoculated *Nostoc* to the total algal biomass. However, because the inoculated strain was dominant, it can be concluded that the dilution of ^{15}N was due mainly to N_2-fixation by *Nostoc*. After 3 weeks, most of the BGA in the pots with surface-applied *Nostoc* disappeared and green algae became dominant. In the other treatments, only green algae developed during the first week; after 3 weeks, BGA other than *Nostoc* appeared.

Analysis of variance showed that the method of application of BGA and ammonium sulfate had a significant effect on the recovery of ^{15}N from rice and weeds at the panicle initiation stage (48 days after transplanting) (Table 2.4) N applied on the soil surface was less available to the plant than N that had been incorporated. The difference between surface-applied BGA and ammonium sulfate was not significant. In contrast, the availability of N from the incorporated ammonium was higher that of N from the incorporated BGA. The higher availability of N from the incorporated materials was confirmed by measurements performed at harvest time (Table 2.5). Analysis of variance also showed a significant effect of the source of nitrogen on the recovery of ^{15}N in plants, indicating that algal N was slightly less available to the rice crop than ammonium sulfate. The patterns of ^{15}N distribution in grain, straw, roots, and weeds were identical in the 4 treatments. Because the amount of total ^{15}N recovered from the plants was almost the same at panicle initiation (PI) and at

Table 2.4. Recovery of ^{15}N from rice shoots, and roots and weeds at PI stage of the first rice crop[a] – pot experiment.

Treatment	Total Plant N (mg pot^{-1})	Recovery of ^{15}N (%) in plants (1st crop)		
		Shoots	Roots and weeds	Total
Surface-applied BGA	127 a	10.4 b	4.1 c	14.5 c
Incorporated BGA	132 a	21.2 ab	6.9 ab	28.1 b
Surface-applied $(NH_4)_2SO_4$	145 a	13.1 b	4.3 c	17.4 c
Incorporated $(NH_4)_2SO_4$	122 a	30.9 a	9.7 a	40.6 a

ANOV: Source of variation	F value
N source (N)	4.21^{ns}
Method of application (M)	23.85^{**}
N x M	$1\ 61^{ns}$

[a] In a column, means followed by a common letter are not significantly different at the 5% level.
^{ns} not significant at the 5% level.
** significant at the 1% level.

Table 2.5. Total N and recovery of ^{15}N in plant and soil after one rice crop[a] – pot experiment.

Treatment	Total Plant N (mg pot^{-1})	Recovery of ^{15}N in plants (%)			
		Grain	Straw	Roots and weeds	Total
Surface-applied BGA	127 a	9.0 c	3.0 b	1.8 b	13.8 b
Incorporated BGA	123 a	17.7 b	6.0 a	4.2 a	27.9 a
Surface-applied $(NH_4)_2SO_4$	118 a	11.4 c	3.9 b	2.4 b	17.7 b
Incorporated $(NH_4)_2SO_4$	125 a	24.6 a	7.8 a	4.3 a	36.7 a

ANOV: Source of variation	F value
N source (N)	32.97^{**}
Method of application (M)	221.7^{**}
N x M	4.5^{ns}

Treatment	Recovery of ^{15}N in soil (%)			Total in plant and soil
	0-2 cm	2-15 cm	0-15 cm	
Surface-applied BGA	30.8 a	28.3 b	59.1 ab	72.9 b
Incorporated BGA	24.0 a	39.6 a	63.6 a	91.4 a
Surface-applied $(NH_4)_2SO_4$	31.1 a	19.1 b	50.2 b	67.9 b
Incorporated $(NH_4)_2SO_4$	27.3 a	25.1 b	52.4 b	88.9 a

ANOV: Source of variation	F value	
N source (N)	9.65^{*}	1.12^{ns}
Method of application (M)	1.05^{ns}	30.27^{**}
N x M	1.27^{ns}	1.14^{ns}

[a] In a column, means followed by a common letter are not significantly different at the 5% level.
^{ns} not significant at the 5% level.
** significant at the 1% level.

maturity, it can be stated that most of the ^{15}N was absorbed before PI and then translocated from the roots and shoots to the grains, and that the ^{15}N concentration in the grains was highest at maturity. Total nitrogen content of rice did not differ among treatments. But the content of ^{15}N remaining in the soil was highly variable. More ^{15}N from incorporated BGA's nitrogen than ^{15}N from incorporated ammonium sulfate remained in the soil after harvest (Table 2.5), reflecting the plant's lower uptake of BGA's nitrogen than of ammonium sulfate nitrogen. In all treatments, total recovery was significantly lower than 100% (Table 2.5). No significant difference in total ^{15}N recovery was observed between BGA and ammonium sulfate applied similarly, indicating that BGA may be as susceptible to nitrogen losses as ammonium sulfate.

After 2 crops, the total recovery of ^{15}N was higher in the BGA-treated pots than in the ammonium-treated pots (Table 2.6). This is in agreement with the higher ^{15}N content after the first crop, in soils where BGA were applied (Table 2.5).

Results of the pot experiment are summarized in Table 2.7. The recovery of 15N from 2 rice crops was 20% with surface applications of either BGA or ammonium sulfate. The availability of BGA's nitrogen to rice was almost comparable to that of ammonium, when the 2 crops were taken into account. After 2 crops, total recovery of ^{15}N was higher from BGA than from ammonium sulfate, mainly because of a lower recovery of ^{15}N in soils and plants after the second crop in ammonium sulfate-treated pots. Results from both first and second crops also confirm the beneficial effect of the incorporation of fertilizers.

2.5.1.3 Availability of BGA-N to the rice plant (Field experiment)

The soil used contained 0.177% N with 0.367±0.02 (std. deviation of 6 samples) atom% ^{15}N. About 10 days after the *Nostoc* application, an algal film covered almost the whole surface of both plots. Microscopic observation of algae showed *Nostoc* as the dominant BGA together with *Anabaena* and unicellular BGA as associated species, suggesting that the inoculated *Nostoc* was able to establish in the field. Eighteen days after the algal application, floating BGA masses were collected from 5 sites in the frames for ^{15}N analysis. Contents were 3.84 atom % excess in the surface-applied treatment

Table 2.6. Total nitrogen and recovery of ^{15}N in the plants and soil after two rice crops[a] – pot experiment

Treatment	Total N	Recovery of ^{15}N(%)					
		In plants (2nd crop)				In soil (total) (0-15cm)	Total in plant and soil
		Grain	Straw	Roots and weeds	Total		
Surface-applied BGA	77.2 ab[1]	4.4 ab	1.6 a	1.1 b	7.1 ab	57.3 a	64.4 a
Incorporated BGA	79.3 a	4.6 a	1.5 a	2.4 a	8.5 a	57.7 a	66.2 a
Surface-applied $(NH_4)_2SO_4$	56.8 c	2.0 c	0.7 b	0.6 c	3.3 c	30.9 b	34.2 b
Incorporated $(NH_4)_2SO_4$	65.0 abc	3.2 bc	1.1 ab	1.4 b	5.7 b	41.6 b	47.3 b
ANOV:							
Source of variation						F value	
N source (N)					35.66**	71.96**	77.07**
Method of application (M)					10.37*	4.81ns	7.03*
N x M					5.79*	4.26ns	3.72ns

[a] In a column, means followed by a common letter are not significantly different at the 5% level.
ns not significant.
* significant at the 5% level.
** significant at the 1% level.

Table 2.7. Recovery of ^{15}N after two crops of rice in soil and plants – pot experiment.

Treatment	Recovery of 15N (%)[a]					Loss of ^{15}N (%)
	In plant			In soil After 2 crops	Total in plant and soil	
	1st crop	2nd crop	Total			
Surface-applied BGA	13.8 b	7.1 ab	20.9 b	57.3 a	78.2 b	21.8
Incorporated BGA	27.9 a	8.5 a	36.4 a	57.7 a	94.1 a	5.9
Surface-applied $(NH_4)_2SO_4$	17.7 b	3.3 c	21.0 b	30.9 b	51.9 c	48.1
Incorporated $(NH_4)_2SO_4$	36.7 a	5.7 b	42.4 a	41.6 b	84.0 a	16.0
ANOV:						
Source of variation				F value		
N source (N)	31.97**	35.66**		71.96**	29.0**	
Method of application (M)	221.7**	10.36*		4.81ns	50.0**	
N x M	4.56ns	5.79*		4.26ns	5.7*	

[a]In a column, means followed by a common letter are not significantly different at the 5% level.
ns not significant.
* significant at the 5% level.
** significant at the 1% level.

and 3.36 atom % excess in the soil-incorporated treatment. Outside the frames where *Nostoc* was not applied, no BGA growth was visible. After 4 weeks, *Spirulina* was the dominant BGA in the plot with surface-applied treatment while *Oscillatoria* and *Nostoc* were the associated species. In the plot with soil-incorporated treatment, *Nostoc* was still the dominant BGA. After 6 weeks, the BGA in both plots disappeared, and weeds and green algae started to grow.

About 20% of ^{15}N from both surface-applied and incorporated Nostoc was absorbed by the first crop (Table 2.8). In both treatments, 3% of ^{15}N was

Table 2.8. Recovery of ^{15}N in plant and soil after the first and second crops of rice – field experiment

Treatment	Recovery of ^{15}N(%)						
	Plant				Soil		
	Straw	Grain	Weeds	Total	0-2 cm	2-15 cm	0-15 cm
1st crop							
Surface-applied	6.7	13.3	3.1	23.1	36.8	9.7	46.5
Incorporated	8.7	12.3	2.5	23.5	32.1	11.5	43.6
95% confidence limit	2.8	2.7	-	3.3	5.8	3.3	6.7
2nd crop							
Surface-applied	0.59	1.78	1.51	3.88	23.8	7.8	31.6
Incorporated	1.01	2.02	0.26	3.29	14.1	18.7	32.8
95% confidence limit	0.36	0.70	-	1.00	3.4	4.2	5.4

recovered in the weeds. The ^{15}N remaining in the soil was on the average, the same in the 2 treatments (44 and 46%). A downward migration of the surface-applied algal material to the 2-15 cm soil layer may have been caused by the action of the soil microfauna and macrofauna and the mechanical effects of buffeting rains (mixing action and percolation) that accompanied two typhoons, one of which occurred just after the application of the algal material. The high concentration of ^{15}N in the surface soil (0-2 cm) cannot be adequately explained by the motility of hormogonia from the incorporated algal material but also be due to an imperfect mixing related to the floating property of the dried algal material. Because the ^{15}N contents of algal films that developed 18 days after the application were similar in the 2 treatments, it is probable that homogenization took place early in the growth cycle.

Recoveries of ^{15}N from plants and soil after 1 crop were approximately 65% in both surface-applied and incorporated BGA treatments (Table 2.8). Recoveries of ^{15}N from the second crop of rice and soil were similar in the two treatments (Table 2.8). Recovery of ^{15}N from rice and soil after 2 crops was about 60% in both treatments (Table 2.9). Because soil samples were not taken below 15 cm from the soil surface, and because the disturbance caused by the typhoons induced appreciable losses of algal material through leaching, some amounts of ^{15}N may not have been recovered. The absence leaching losses from the pots is the reason for the higher ^{15}N recovery from the pots particularly in the subsurface soil than from the field (Table 2.5 and 2.8).

2.5.2 Plant-associated BNF (ARA) in 16 rice varieties over a growth cycle and related plant parameters

Table 2.9. Recovery of ^{15}N in plant and soil after 2 crops of rice – field experiment

Treatment	Recovery of ^{15}N (%)					
	In plant			In soil (0-15 cm) after 2 crops	Total in plant and soil (0-15 cm)	^{15}N unrecovered (%)
	1st crop	2nd crop	Total			
Surface-appled	23.1	3.9	27.0	31.7	58.7	41.3
Incorporated	23.5	3.3	26.8	32.8	59.6	40.4

Table 2.10. F values from ANOV of ARA per plant of 8 long-duration and 8 short-duration varieties during the WS.

Days after transplanting	F (%CV)	
	Long-duration	Short-duration
40	1.21 (61)	0.706 (47)
55	1.55 (36)	4.12* (2.5)
74	2.43** (29)	-
81	5.31** (20)	-
Heading	5.70** (28)	3.02* (27)

*, ** significant at the 5% and 1% level respectively.

2.5.2.1 Trends of ARA and plant growth characters

Figure 2.1 shows schematic trends of ARA, shoot and root dry weights, leaf area and tiller number in the long- and short-duration varieties, showing a peak at heading. On the other hand, the root dry weight and leaf area of the long-duration varieties declined before heading stage. Panicle primordial initiation occurred before the maximum number of tiller stage in the short-duration varieties and after the maximum number of tiller stage in the long-duration varieties.

2.5.2.2 Varietal differences in ARA

During the WS, when ARA was measured at different stages of rice growth, it was at heading that ARA per plant differed significantly among the long- and short-duration varieties. The coefficient of variation (CV) of ARA measurements among blocks was very high during the early rice growth stages (36-61%). At later stages, the CV was less than 30% (Table 2.10). During the DS, ARA was measured only at heading. The CV was below 17% in both long- and short-duration groups and significant differences on a per plant basis were found among the long-duration varieties. ARA per plant dry weight basis, on the other hand, showed significant differences among varieties of both groups and seasons (Table 2.11).

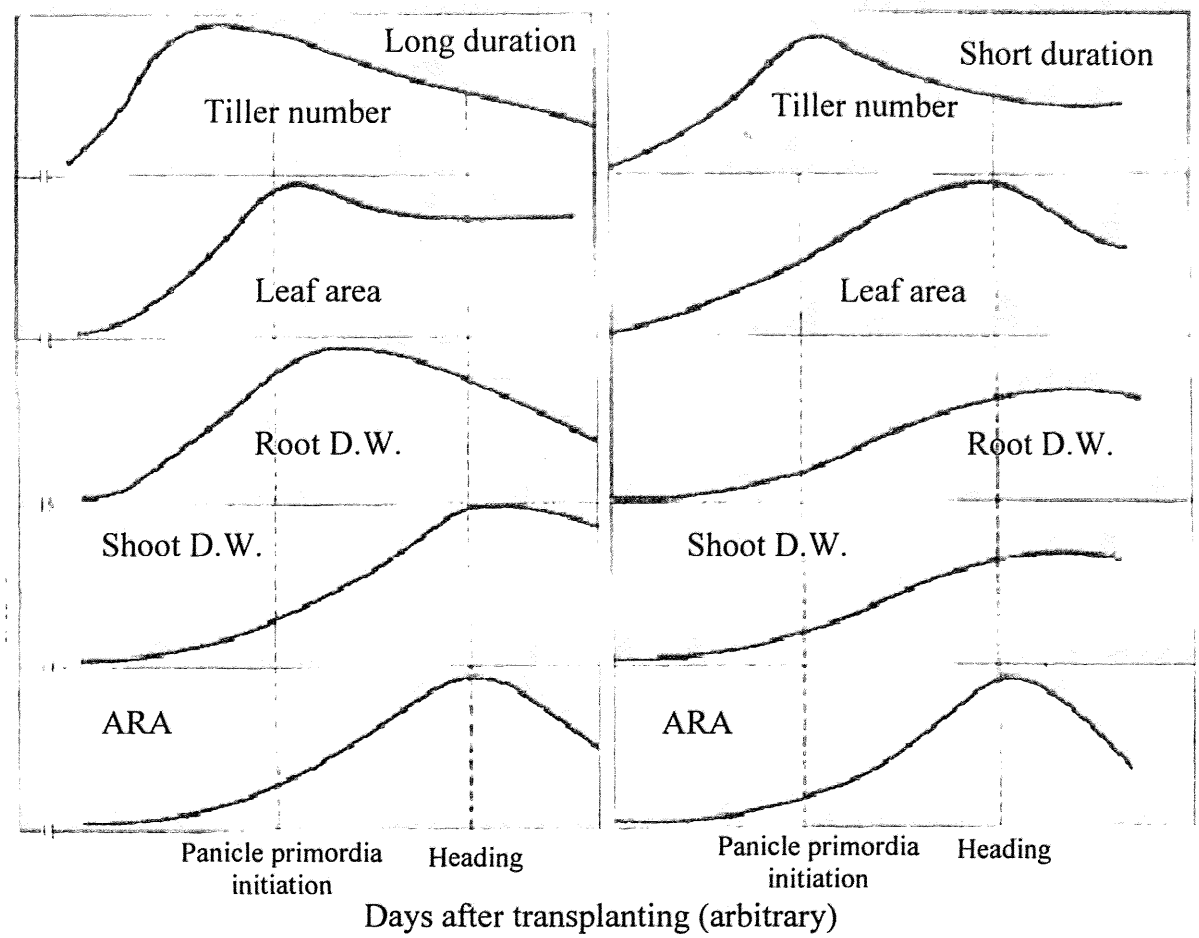


Fig. 2.1 Trends of plant-associated ARA and plant characters in long- and short-duration varieties. Trends are based on 8 long- and 8 short-duration varieties measured during the wet season.

Long –duration varieties had higher ARA than the short-duration varieties, except IR50 which had an ARA similar to that of some of the long-duration varieties. IR50 also had the highest specific activity (ARA per unit plant dry weight) among the short-duration varieties in both seasons. Among the long-duration varieties, IR42 exhibited the highest ARA expressed on per plant or per plant dry weight basis. IR20 also ranked high in terms of ARA per unit dry weight. Line IR21912-56-3-1-2-2 ranked low in both seasons (Table 2.11).

2.5.2.3 Correlations of ARA with plant growth characters

Table 2.12 shows single and partial correlation coefficients of ARA per plant at heading with several plant characters. ARA had a significant positive correlation with root and total plant dry weight at heading and total plant dry weight at harvest in both seasons. This is the reason why ARA per plant dry weight was calculated to compare varieties. ARA correlated with shoot dry

Table 2.11. ARA per plant and per plant dry weight at heading stage of several long- and short-duration varieties during the wet and dry seasons.

Variety	Wet season ARA		Dry season ARA	
	μmol C_2H_4 $plant^{-1}$ $6\ h^{-1}$	nmol C_2H_4 g^{-1} $6\ h^{-1}$	μmol C_2H_4 $plant^{-1}$ $6\ h^{-1}$	nmol C_2H_4 g^{-1} $6\ h^{-1}$
IR42	12.7	219	7.8	218
Rodjolele	8.9	125	5.5	104
IR20	7.8	186	4.9	205
Peta	7.8	119	7.7	168
IR13540	6.0	116	3.2	130
IR22082-4-1-2	5.7	110	5.6	181
IR18349-135-2-3-2-1	4.5	90	4.7	149
IR15314-30-3-1-3	5.2	114	5.2	133
ANOV (F)	5.7**	4.9**	4.8*	7.7**
Standard Error	1.21	20.6	0.45	15.1
IR50	5.2	183	5.1	231
IR13240-82-2-3-2-3-1	5.2	129	3.7	157
IR13427-45-31-2-2-2(3107)	4.5	113	3.1	132
IR36	4.3	105	4.0	178
IR31802-48-2-2-2-3189	3.5	91	2.9	146
IR9729-67-3	3.3	111	3.9	209
IR21912-56-3-1-2-2	2.6	92	3.0	143
IR19728-9-3-2-3-3	2.5	86	3.7	215
ANOV (F)	3.02*	4.65**	2.6	5.12*
Standard error	0.61	14.5	0.73	16.8

*. ** significant at the 5% and 1% level respectively.

Table 2.12. Single and partial correlation coefficients between ARA and plant characters at heading.

ARA vs.	Constant factor	Correlation coefficient	
		Wet season	Dry season
Root dry weight		0.864**	0.702**
	Shoot dry weight	0.735**	0.368
Shoot dry weight		0.722**	0.677**
	Root dry weight	0.362	0.277
Total dry weight		0.733**	0.674**
	Leaf area	0.705**	0.064
Leaf area		-0.333	0.729**
	Total dry weight	-0.185	0.381
Total dry weight at maturity		0.729**	0.518*

*, ** significant at the 5% and 1% level respectively

weight at heading in the WS but there was no correlation when the root dry weight was kept constant in both seasons. On the other hand, ARA correlated with the root dry weight at heading when the shoot dry weight was kept constant in the WS only. ARA also positively correlated with leaf area in the DS.

2.5.2.4 Harvest index, nitrogen harvest index, and nitrogen remobilization efficiency and their relation to ARA.

Grain yield ranged from 4 to 7 t ha^{-1} for the long duration varieties and 2.5 to 5 t ha^{-1} for the short duration varieties in both the dry and wet seasons. Harvest index ranged from medium (0.4-0.5) to high (>0.5) in IR varieties or lines but was low (<0.4) in traditional varieties, Rodjolele and Peta (Table 2.13). In most of the

Table 2.13. Grain and total dry matter yield t ha^{-1}, and harvest index (HI) of long- and short-duration varieties

Variety	Wet season			Dry season		
	Grain yield	Total dry matter	HI	Grain yield	Total dry matter	HI
Long duration						
IR42	6.9	13.8	0.50	3.4	8.8	0.38
Rodjolele	4.2	13.4	0.32	4.2	12.2	0.34
IR20	6.6	11.4	0.58	4.1	8.2	0.50
Peta	5.8	15.5	0.37	3.2	7.8	0.39
IR13540	6.9	13.7	0.50	6.1	13.7	0.45
IR22082-4-1-2	4.1	8.8	0.47	3.2	6.6	0.50
IR18349-135-2-3-2-1	4.8	11.1	0.44	4.4	8.8	0.49
IR15314-30-3-1-3	4.8	10.9	0.44	3.6	7.1	0.51
Standard error	0.41	0.67	0.018	0.43	0.92	0.02
Short duration						
IR50	3.6	7.3	0.50	2.8	6.2	0.45
IR13240-82-2-3-2-3-1	4.8	9.1	0.53	3.0	6.8	0.48
IR13427-45-31-2-2-2(3107)	3.1	7.0	0.44	3.3	6.8	0.48
IR36	4.1	9.1	0.44	3.4	6.6	0.51
IR31802-48-2-2-2-3189	3.4	6.6	0.52	2.6	5.8	0.45
IR9729-67-3	3.7	7.9	0.43	3.3	7.4	0.44
IR21912-56-3-1-2-2	4.3	8.3	0.52	3.5	7.5	0.46
IR19728-9-3-2-3-3	4.5	9.1	0.49	3.2	7.7	0.41
Standard error	0.40	0.52	0.026	0.30	0.57	0.29

varieties, grain yield and total dry matter were lower in the DS than in the WS except for the varieties Peta, IR15314-30-3-1-3, IR13427-45-31-2-2-2 and IR9729-67-3. IR42 gave the highest yield in the WS, but the yield decreased in the DS due to severe attack by tungro virus.

Nitrogen harvest index (NHI) and NRE of the varieties were also determined during the WS (Table 2.14). NHI was significantly different among the long- and short-duration varieties while NRE was different only among the short-duration varieties. The long-duration varieties had a wider range of NHI (0.47-0.81) than

Table 2.14. N harvest index (NHI) and N remobilization efficiency (NRE) of long- and short-duration varieties during the WS.

Variety	NHI	NRE
Long duration		
IR42	0.65	0.66
Rodjolele	0.47	0.56
IR20	0.69	0.65
Peta	0.52	0.52
IR13540	0.62	0.65
IR22082-4-1-2	0.80	0.77
IR18349-135-2-3-2-1	0.71	0.70
IR15314-30-3-1-3	0.70	0.68
ANOV (F)	4.18**	2.54
Standard error	0.018	0.048
Short duration		
IR50	0.70	0.75
IR13240-82-2-3-2-3-1	0.73	0.68
IR13427-45-31-2-2-2(3107)	0.60	0.60
IR36	0.64	0.60
IR31802-48-2-2-2-3189	0.73	0.74
IR9729-67-3	0.66	0.66
IR21912-56-3-1-2-2	0.70	0.63
IR19728-9-3-2-3-3	0.69	0.56
ANOV (F)	2.4*	3.06*
Standard error	0.24	0.042

*, ** significant at the 5% and 1% level respectively

the short-duration varieties (0.6-0.73) due to the lower NHI of traditional varieties Peta and Rodjolele. Peta and Rodjolele also had lower NRE's than the other varieties. ARA per plant was not correlated with HI, NHI and NRE.

2.6 Discussion

2.6.1 Implications for agronomic use of BGA in rice cultivation

Total algal biomass evaluations in rice fields range from a few kg ha^{-1} to 58 t fresh weight ha^{-1} or 500 kg dry weight. Reported N_2-fixing algal biomasses range within the same limits (Roger and Kulasooriya, 1980). Assuming a maximum biomass of 500 kg dry weight ha^{-1} and using average N content for field samples (2.52%) and laboratory cultures (5.0%), it appears that the potential N contribution of a N_2-fixing bloom is around 13 to 25 kg N ha^{-1}.

Using average values for dry matter, ash, and N contents, it can be estimated that the average biomass corresponding to 10 kg N is about 8 t fresh weight. This is equivalent to a continuous layer of 0.8 mm algal material over 1 ha of a rice field. In other words, an algal bloom of agronomic significance is visible to the naked eye.

When looking at BGA as a source of nitrogen for rice, the C:N ratio is a major factor determining N mineralization. Extreme C:N values observed in laboratory cultures were 4.3 and 13, which confirm that variability is larger in artificial conditions than in nature. The C:N ratios from field samples were less variable with an average of 8.5. This indicates that BGA have better nitrogen availability than organic fertilizers such as farmyard manure and green manures.

2.6.2 Availability of N from BGA in rice paddy soils

The algal material spread in the field corresponded to 29 kg N ha^{-1}, 290 kg dry weight ha^{-1}, and 13 t fresh weight ha^{-1}. This quantity of algal material may be considered equivalent to that of a dense algal bloom (Roger and Kulasooriya, 1980).

The pot experiment conducted in the greenhouse was protected from environmental disturbances and therefore permitted a good comparison between surface-applied and incorporated treatments. However, in the pots, prevention of losses through leaching led to an overestimation of nitrogen availability to the plant. The field experiment was subjected to severe environmental disturbances, including two typhoons. Therefore, surface-applied and incorporated material could not be compared and important losses from downward movement and seepage decreased N availability to the rice plant and the total N recovery from rice and soil up to a depth of 15 cm.

The two treatments (surface-applied and incorporated) were selected to represent two prevailing situations in the field. Under natural conditions, N_2-fixing algal blooms develop any time throughout the cultivation cycle; however, early growth is rare and BGA seem to develop preferentially during the second part and at the end of the growth cycle of rice (Roger and Kulasooriya, 1980).

When the algal bloom develops later in the cycle, most of the algal material will dry on the surface of the soil, be incorporated by plowing, and start to decompose only at the beginning of the next growth cycle. This sequence is similar to the situation where dried BGA were incorporated. From results of the pot and field experiments, it appears that the availability of nitrogen from BGA incorporated into the soil ranged between 23 and 28% for the first crop and between 27 and 36% for the first and second crops (Tables 2.7 and 2.9).

If the algal bloom develops early in the cycle, decomposition of lytic microorganisms and grazing by aquatic fauna, are likely to occur later during the

same cycle, thereby making nitrogen available in the floodwater and on the soil surface. This situation, which is infrequent under natural conditions occurs when paddy fields are inoculated by algae and is somewhat similar to that in the treatments where dried BGA were surface-applied, since an algal bloom also developed early in the cycle, but different in that the decomposition started at the beginning of the growth cycle. That may have led to an overestimation of the availability of algal nitrogen to the current rice crop. From the results of the pot and field experiments, it appears that the availability of nitrogen from BGA spread on the soil surface ranged between 14 and 23 % for the first crop and between 21 and 27 % for both the first and second crops.

In the field experiment, climatic disturbances in addition to a low depth of sampling did not permit balance calculations and estimation of losses. In the pot experiment, apparently more nitrogen was lost from ammonium sulfate than from BGA after two crops. However, applied BGA multiplied causing an increase of the total nitrogen in the pot and a dilution of algal ^{15}N. Therefore, although ^{15}N was representative of the total N balance in the ammonium sulfate-treated pots, ^{15}N balance in the BGA-treated pots underestimated losses of total nitrogen as well as availability of algal nitrogen to the plant.

Availability of algal nitrogen reported by Wilson et al. (1980), from a pot experiment, was almost twice as high as that which we measured under similar experimental conditions. The reason for this discrepancy is certainly related to the nature of the algal material, the method of preparation, and the nature of the strain. Wilson et al. (1980) used an algal material collected directly from the flask culture and blended after suspension in distilled water. Therefore, it can be suspected that this material was highly susceptible to decomposition because it consisted mainly of vegetative cells, a part of which may have been damaged by blending. In contrast, the material used in this study consisted mainly of vegetative cells in dormancy and of akinetes and was, therefore, much less susceptible to decomposition. This hypothesis is in agreement with the results of a preliminary pot experiment where the same Nostoc strain directly collected from the carboy culture was used. When this fresh material, composed mainly of vegetative cells, was incorporated, about 38% of the ^{15}N was recovered in the first crop instead of 28% when dried material was used.

In agreement with Wilson et al. (1980), an enhanced availability of BGA-N was observed by incorporation. This may be due to an accelerated decomposition of BGA

in the anaerobic soil layer and a better proximity of the released nitrogen to the roots. The beneficial effect of deep placement of nitrogen fertilizers due to the decrease of gaseous nitrogen losses was confirmed in this study.

The pot experiment demonstrated that for the first crop, algal nitrogen was less available than ammonium sulfate but for two crops its availability was very similar to that of ammonium sulfate indicating the slow release nature of BGA-N, a finding which agrees with the cumulative effect of algal inoculation (Roger and Kulasooriya, 1980). However, its very low C:N ratio (5-6) gives it a better nitrogen availability than that of organic fertilizers such as FYM.

2.6.3 Varietal differences in rice plant-associated N_2-fixation (ARA)

Acetylene-reducing activity associated with rice is measured at heading because it is at this stage that ARA was found to be highest and the coefficient of variation among replicates is lowest. It is also at heading stage that varietal differences in rice plant-associated ARA can be detected. Activities ranged from 0.4 to 2 μmol C_2H_2 $plant^{-1}$ h^{-1}. Assuming that ARA measured at heading lasts 50 days, a C_2H_2/N_2 ratio of four, and a plant density of 25 m^{-2}, N_2-fixing rate would be 1-5 kg N ha^{-1} $crop^{-1}$. Extrapolations from ^{15}N experiments range from 1.3 to 7.2 kg ha^{-1} $crop^{-1}$ (Roger and Watanabe 1984). A modified plant sampling procedure was developed to detect varietal differences in rice-plant associated ARA. In this method, 2 plants per block from 3 blocks for 3 consecutive days or a total of 18 plants per variety were sampled. By sampling plants this way, day-to-day variation in ARA was taken into account, and the plant sampling size was increased from 6 to 18.

Plant ARA among the long- and short-duration varieties peaked at heading, but the trends of root biomass in the long- and short-duration varieties differed. There was a peak followed by a decline before heading in the long-duration varieties and a peak at heading in the short-duration varieties. The incidence of maximum ARA at heading stage may be due to a) a rapid depletion of available N in the soil; b) increased supply of plant photosynthates to roots; c) increased transport of atmospheric nitrogen to the roots through the aerenchyma at heading. It is also possible that in at least the long-duration varieties, ARA is enhanced by an increased availability of carbon compounds due to partial degradation of plant roots at heading. However, if the decline of roots were a possible cause for the peak of ARA, then a peak should appear after the decline of root biomass in the short-duration varieties. The absence of such a

peak rules out the dominant role of degraded root but suggests the supply of photosynthates as a more dominant factor affecting ARA.

The field used in this experiment was dry-fallow for more than 3 years resulting in an accumulation of nutrients during the fallow period which consequently might have become available for the first crop. This may explain the higher plant biomass and ARA on a per plant basis in most of the varieties grown in the first crop (WS). The specific ARA among varieties was higher in the dry season crop. This may be associated with the higher plant photosynthetic efficiency in the DS resulting in increased supply of photosynthates to the rhizosphere.

Among the plant characters, root biomass appears to be the most important factor affecting plant ARA as also indicated by partial correlation coefficients. ARA did not correlate with the shoot biomass when the root biomass was kept constant but correlated with the root biomass even when the shoot biomass was kept constant.

IR42 and IR50 consistently showed the highest ARA per plant and unit plant dry weight among the long- and short-duration groups in both seasons. IR42 has also earlier been found to support high N gains and N_2 fixation (IRRI, 1983; Ladha et al., 1986) and perform better in soil with low N fertility (De Datta, 1984). IR50 is a unique, short duration variety with a high specific ARA comparable to IR42, a long-duration variety with a very high ARA. Such results from a limited number of varieties indicate the variability in specific N_2-fixing activity and suggests the importance of selecting this trait for transfer into high yielding varieties.

If ARA per plant dry weight were constant, the major determining factor for ARA would be plant biomass. However, significant differences were found in specific ARA. Thus, the relative contribution of biological N_2-fixation (ARA) to plant N is a function of plant weight and ARA per plant. Considering the contribution of N_2-fixation to the plant's N nutrition, ARA per plant dry weight may be a better index for evaluating plant ARA.

Despite the high plant biomass of traditional varieties, neither ARA per plant nor ARA per plant dry weight was high. Inefficiency in the transport of photosynthates to the developing grain in tall varieties also appears to be related to the inefficiency of supporting N_2-fixation at heading. There was no correlation of ARA per plant or per plant dry weight with HI, NHI or NRE. In general, NHI equaled NRE suggesting little N input to plant or loss from heading to harvest. It is desirable to find rice plants with the trait to stimulate N_2-fixation among those with a higher HI or NHI.

CHAPTER 3
EFFECTS OF INORGANIC AND ORGANIC FERTILIZATION ON HETEROTROPHIC BIOLOGICAL NITROGEN FIXATION

3.1 Summary

The relative contributions of N_2 fixed by heterotrophic diazotrophic bacteria depend on the availability of substrates from straw decomposition and on environmental pressures. Straw application enhanced heterotrophic and plant-associated N_2-fixation. Both surface applied and incorporated rice straw (chopped, 5 t ha^{-1}) applied to the field 2 weeks before transplanting stimulated plant-associated N_2-fixation as measured by acetylene-reducing activity (ARA) in IR42 but not in IR50. The ARA of soil under dark conditions (heterotrophic N_2-fixation) showed maximum activity during the early period of straw decomposition. It increased within a few days and declined almost similar to that of the control, 4-5 weeks after straw incorporation. The ARA of unammended soil remained almost constant. Straw application increased the population of total and N_2-fixing heterotrophs in the soil. Higher bacterial population was observed with surface application of straw as compared with incorporated straw.

Although it is possible to obtain moderate yields without any N input due to BNF, it is more often necessary to add inorganic and/or organic N inputs to increase yields. The N_2-fixing activities associated with rice plants obtained from field experiments using different N sources were measured by a modified short-term acetylene reduction assay. Besides, urea, grain legume green manure (*Vigna radiata* and *Dolichos lablab*), non-grain legume green manure (*Sesbania* and *Crotoloria*), non-legume green manure (*Azolla*), and rice straw were also used as N sources. Nitrogen fixation per plant (or per unit area) was either enhanced or not affected by the application of inorganic N and organic fertilizers. However, when expressed on a per unit plant dry weight basis, the ARA were generally lower except in cases where some legume green manures such as *V. radiata* and *Crotolaria* were incorporated. Possible mechanisms of how rice plant associative N_2-fixation is affected by different N fertilizers are discussed.

3.2 Introduction

It has been shown that N_2-fixation by heterotrophic and non-heterotrophic bacteria in the submerged soil is markedly stimulated by straw (Yoneyama et al., 1977; Rao, 1976; Wada et al., 1979; Reddy and Patrick, 1979; IRRI, 1981; Lynch and Harper, 1983). All of

these were small-scale experiments conducted in the laboratory or greenhouse and hence, may not have much bearing in the field. In laboratory culture systems, inoculation of straws with cellulolytic and diazotrophic microorganisms has resulted in significant increases in N_2 fixation in comparison with uninoculated controls and gains of N of up to 72 mg N fixed g^{-1} straw consumed have been obtained, indicating the potential of inoculation to improve N gains in composts that can then be used as biofertilizers (Roper and Ladha, 1995). Soils on the other hand, contain established, indigenous microbial populations which tend to exclude inoculant microorganisms by competition. As a consequence, improvements in straw-associated N_2-fixation in soils have been achieved mostly by specific straw-management practices which encourage microbial activity by straw-decomposing and N_2-fixing microorganisms. Matsuguchi (1979) and Matsaguchi and Yoo (1981) conducted field experiments to study the effects of straw and compost, with and without inorganic N fertilizer on N_2-fixation (acetylene reduction) in wetland rice fields of Japan. They reported a substantial stimulation of acetylene-reducing activity in the plough layer with the incorporation of 10 t straw ha^{-1}. However, little has been reported on the effects of different modes of straw application on N_2-fixation and microbial population throughout the growth cycle in tropical rice fields prior to this study.

Moderate but sustainable yields of wetland rice can be obtained with no input of N fertilizer (Koyama and App, 1979). This has been attributed to plant-associative and free-living biological nitrogen fixation (Watanabe, 1986). However, additional inorganic and/or organic N is required in order to obtain higher yields. Several studies have illustrated almost complete and long-lasting inhibitory effects of N fertilizer on the nitrogen-fixing (C_2H_2-reducing) activity of free-living cyanobacteria (Roger and Kulasooriya, 1980). On the other hand, a critical and systematic study on the effects of combined N on rice plant-associative N_2-fixation was lacking.

3.3 Objectives

The objectives of this study were to determine:

a. the effects of two methods of straw application on acetylene-reducing activity (ARA) associated with the rice plant and non-rhizospheric soil, and on the N_2-fixing bacterial populations; and
b. the effects of inorganic N and organic fertilizer management on rice plant-associated N_2-fixing activity (ARA).

3.4 Materials and Methods

3.4.1 Determination of the effects of 2 modes of straw application to a wetland rice field on acetylene-reducing activity (ARA) associated with the rice plant and non-rhizospheric soil, and on the N_2-fixing bacterial populations.

3.4.1.1 Field experiment

The experiment was laid-out in a split-plot design with 4 replications for 3 mainplot treatments: a) control, b) surface application of straw, and c) incorporation of straw. There were 2 subplots (varieties). Each subplot was 8.5 x 4.7 m in size. The field was ploughed and harrowed 4 weeks before transplanting. Straw pieces (10-15 cm in the dry season (DS) and about 5 cm in the wet season (WS) were surface-applied or incorporated 2 weeks before transplanting. Before transplanting, 20kg P ha^{-1} as $KHPO_4$ was applied and incorporated. The straw-treated plots received 5t straw ha^{-1} cut into 10- to 15-cm pieces. In surface-applied plots, the straw pieces were uniformly spread. A flood water level of about 3-5 cm was maintained in all plots.

Two cultivars of *Oryza sativa*, IR42, a long-duration variety and IR50, a short-duration variety, were used. Rice seedlings were transplanted without incorporating straw in the surface-applied plots.

3.4.1.2 Plant-associated ARA

As described in section 2.4.2.2.

3.4.1.3 Soil ARA

Six soil cores, 7.2 cm inside diameter (i.d.) and 13 cm long, were taken from each main plot. Two soil cores were combined in one plastic bag making a total of 3 replicate samples per plot. Each bag was sealed and 1 liter of a mixture of 25% C_2H_2 and 0.002% C_3H_8 in air was added through an injection port. Gas samples were then collected 30 min and 24 h after the gas was introduced. The ethylene and propane concentrations were determined by gas chromatography (Lee et al., 1977).

3.4.1.4 Enumeration of bacteria

The total and N_2-fixing heterotrophs were counted 2 days before straw application (DBSA) and at 10, 20, 34, 54, and 90 days after straw application (DASA). Six soil cores, 10cm i.d., 13 cm long, per block (a total of 2 blocks per treatment with and without straw amendment) were collected. From each core, about 1 cm surface soil was removed and the soil samples of each treatment were pooled (total of 12 cores per treatment). Soil was passed through a 2-mm mesh sieve to remove pebbles, weeds, roots, and partially decomposed straw pieces, if any, and then mixed thoroughly. Duplicate 100-g soil samples and also 50 g of macerated straw fragments were serially diluted in 0.001M phosphate buffered water (pH 7.1).

The total heterotrophs were enumerated by plating 0.1 ml aliquots of appropriate dilutions unto TSA plates [0.1% tryptic soy broth (Difco) plus 1.5% Noble Agar (Difco) and incubating these at 32°C for 10 days. N_2-fixing heterotrophs were counted by randomly picking up about 100 colonies per replicate per treatment from the TSA plates and putting them into a glucose yeast-extract, semisolid medium. The ARAs of the isolates were determined using the method described by Watanabe et al.(1979).

3.4.1.5 Determination of plant dry weight and yield

As described in section 2.4.2.3

3.4.2 Determination of the effect of inorganic N and organic fertilizers on rice plant-associated N_2-fixation

3.4.2.1 Field experiments

Rice plants were obtained from the irrigated wetland rice fields of IRRI. The soil (Maahas clay) is an isothermic clayey mixed Aquic Tropudalf (pH 6.2; organic matter, 2.9%; total N, 0.15%; and CEC, 49meq $100g^{-1}$ soil). The experimental details are shown in Table 3.1.

Basal treatment of inorganic N fertilizer involved application of urea 1 day before transplanting and a topdressing at the panicle initiation (PI) stage. Green manure (GM) was grown in the same field before rice either in continuously flooded (expt. 3) or in rainfed conditions (expts. 2 and 4). In

Table 3.1 Details of the experiments conducted to determine the effects of inorganic N and organic fertilizer management on rice plant-associated N_2-fixation.

Expt. No.	Treatments[a]	Crop No. in the sequence	Field layout[b]/ subplot size (m^2)	No. of rep-licates	Season[b]	Rice Variety	Time of ARA and plant biomass measurements (days after transplanting)
1	Control-no N, 60 kg N ha^{-1} basal, 40 kg N ha^{-1} basal + 20 kg N ha^{-1} at PI, 70 kg N ha^{-1} basal + 30 kg N ha^{-1} at PI	1&2	RCBD/15	3	WS	IR50	22, 37, 50, 68, and 78(heading)
2	Control-weed free, Weedy fallow, Weedy fallow + urea (70 kg N ha^{-1}), *Crotolaria juncea* incorporated, *Vigna radiata* incorporated, *Sesbania aculeata* incorporated, *Sesbania aculeata* + urea (35 kg N ha^{-1}) *Dolichos lablab* incorporated	3	SPD/30	3	DS	IR54	74,75,76 (3 consecutive days at heading)
3	Control-no N, Urea applied, Azolla incorporated, Sesbania incorporated	3&4	RCBD/49	4	DS	IR54	43, 74 (heading)
4	Different cropping sequences: Fallow (F)-Rice(R)-Maize(M), F-R+farmyard manure-M, F-R+urea(55 kg N ha^{-1})-M, F-R+urea(90 kg N ha^{-1})-M, F-R+urea(125 kg N ha^{-1})-M, *Sesbania aculeata*-R-M, *Vigna radiata*-R-M	3	SPD/2	4	WS	IR54	74, 75, 76 (3 consecutive days at heading)
5	Control-no N, Prilled urea (best split), Urea supergranule, Azolla incorporation, *Sesbania rostrata* incorporation, Rice straw incorporation, Rice straw compost incorporation	10	RCBD/16	4	DS	IR64	32, 47, 70(heading)
6	Control (30 kg N ha^{-1}), Urea (80 kg N ha^{-1}), Chopped straw applied before transplanting + urea (30 kg N ha^{-1}), Long straw applied after transplanting + urea (30 kg N ha^{-1})	3	RCBD/16		DS		33, 63, and 90(heading)

[a] Experiment nos. 3 and 6 received 20 kg P_2O_5 ha^{-1} $crop^{-1}$ as solophos before transplanting and experiment 5, 30 kg. Experiment no. 6 also received 30 kg K_2O ha^{-1} crop-1

[b] RCBD, randomized complete block design; SPD, split plot design; DS, dry season; WS, wet season

experiment 5, the green manures were from another field. *Sesbania* and *Crotolaria* were sown by broadcasting at seeding rates of 40-50 kg ha^{-1} while other legumes were sown at a rate of 30 kg ha^{-1}. Rice seedlings, 20 days old, were transplanted 1 to 7 days after incorporation of organic manure in 20 x 20 cm spacing. The field was then kept flooded. Pesticides were applied as needed.

3.4.2.2 Plant sampling, AR assay, and determination of plant dry weight

As described in sections 2.4.2.2 and 2.4.2.3

3.5 Results

3.5.1 Effects of 2 modes of straw application to a wetland rice field on acetylene-reducing activity (ARA) associated with the rice plant and non-rhizospheric soil, and on the N_2-fixing bacterial populations.

3.5.1.1 Plant and soil ARA

ARA associated with IR42 per plant was significantly higher in the surface-applied and incorporated straw treatments than in the control. When the ARA of IR42 is expressed per unit plant biomass at heading stage, it was significantly higher only in the straw-incorporated treatment. The ARA of IR50 was unaffected by both straw treatments (Table 3.2).

Table 3.2. Effect of straw addition on rice plant-associated ARA at heading stage.

Treatment	ARA μmol C_2H_4 $plant^{-1}$ $6\ h^{-1}$		ARA nmol C_2H_4 g^{-1} plant dry weight $6\ h^{-1}$	
	IR50	IR42	IR50	IR42
Control	3.21	6.86	127	142
Straw, surface-applied	3.73	10.7*	111	158
Straw, incorporated	3.51	10.1*	115	175*

*Significantly different from the control at the 5% level.

Surface-applied straw significantly stimulated soil ARA at 17 and 28 days after straw application (DASA); incorporated straw at 17 DASA (Fig. 3.1). The higher ARA due to the straw treatments was associated with the higher variation of ARA among plots (data not shown). Over time, the ARA of unamended soil remained almost constant.

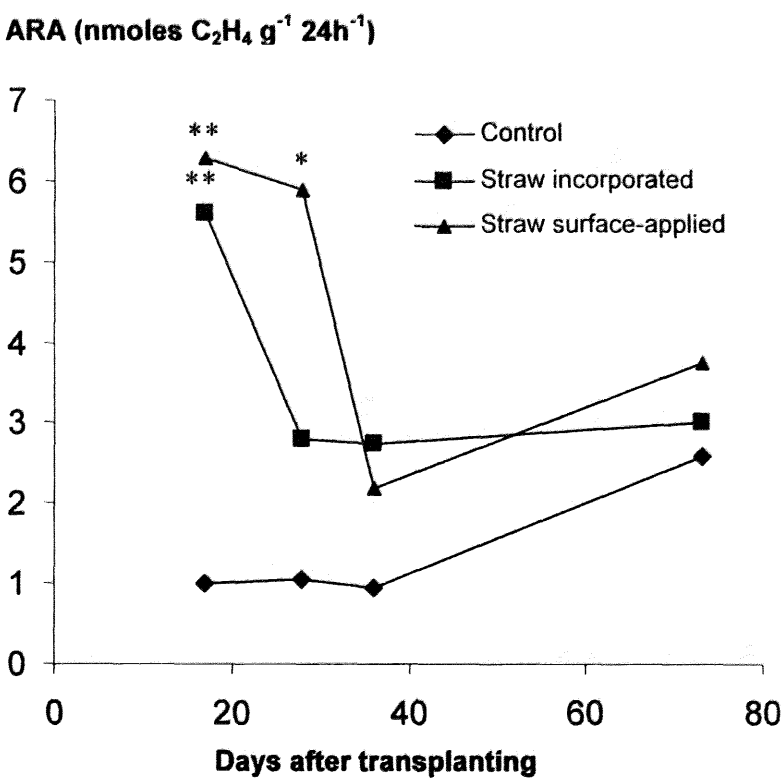


Fig 3.1. Effect of rice straw amendment on ARA of non-rhizospheric weland paddy soil. * and ** indicate significantly higher ARA from control at the 5% and 1% level respectively.

3.5.1.2 Enumeration of total aerobic and N_2-fixing heterotrophs

A high number of total aerobic and N_2-fixing heterotrophs was found to be associated with degrading straw in both surface-applied and incorporated treatments (Tables 3.3 and 3.4). Straw application stimulated bacterial multiplication in the soil, which was more pronounced during the early period of applying straw. Surface-applied straw had a higher bacterial count than incorporated straw.

In all treatments and sampling times, the surface layer of the soil (0-1 cm) had a higher number of total and N_2-fixing heterotrophs than the subsurface or reduced layer (1-15 cm) (Tables 3.3 and 3.4).

3.5.1.3 Plant growth and grain yield

Straw application increased root, shoot, and total plant dry weight at the heading stage in both IR50 and IR42 (Table 3.5 and 3.6). The effect of surface application of straw was more pronounced than that of straw incorporation. On the other hand, both methods of straw application increased the total dry

Table 3.3. Enumeration of total aerobic heterotrophic bacteria as affected by straw application

Days after straw application	Log(10) $CFUg^{-1}$ dry soil or degrading straw[a]		
	Control	Straw incorporated	Straw surface-applied
0-1 cm soil			
-2	6.40	6.60	6.60
10	6.37	6.54	6.94* (8.49)
20	6.20	6.20	6.24 (7.74)
34	6.55	6.57	6.46 (8.26)
54	6.62	6.44	6.57
90	6.34	6.35	6.31
1-15 cm			
-2	6.06	6.08	6.08
10	5.95	6.03 (7.01)	6.18**
20	5.86	5.91 (6.88)	6.10
34	5.93	5.79 (6.94)	6.11
54	6.06	6.13	6.08
90	5.87	5.95	5.98

[a] CFU, colony-forming unit. In parentheses, counts of total N2-fixing heterotrophs associated with degrading straw are given.
*, ** Significantly different from the control at the 5% and 1% level respectively.

Table 3.4. Enumeration of N_2-fixing bacteria as affected by straw application.

Days after straw application	Log(10) $CFUg^{-1}$ dry soil or degrading straw[a]		
	Control	Straw incorporated	Straw surface-applied
0-1 cm soil			
-2	4.85	5.10	5.10
10	5.32	5.39	6.07** (7.80)
20	<4.2	<4.2	<4.24 (6.35)
34	<4.55	4.72	5.08* (7.10)
54	<4.6	4.59	5.41*
90	4.73	<4.3	<4.3
1-15 cm			
-2	4.44	4.58	4.58
10	4.95	4.95 (6.46)	5.61**
20	<3.8	4.06 (6.01)	4.54
34	4.32	3.98 (5.48)	4.50
54	<4.0	4.37	4.47
90	4.18	4.40	<4.0

[a] CFU, colony-forming unit. In parentheses, counts of total N_2-fixing heterotrophs associated with degrading straw are given.
*, ** Significantly different from the control at the 5% and 1% level respectively.

Table 3.5. Effect of straw addition on plant biomass at heading stage

Treatment	Plant dry weight g^{-1} plant					
	Root		Shoot		Total	
	IR50	IR42	IR50	IR42	IR50	IR42
Control	7.9	15.1	16.4	33.4	25.7	48.6
Straw, surface-applied	9.3*	18.6*	24.1*	49.0	33.5*	67.5*
Straw, incroporated	9.2	17.5*	22.2*	41.3	31.4*	58.8

* Significantly different from the control at the 5% level.

Table 3.6. Effect of straw addition on grain and total dry matter yield of IR50 and IR42 at harvest

Treatment	Yield t ha^{-1}			
	Grain		Total dry matter	
	IR50	IR42	IR50	IR42
Control	2.2	1.6	4.5	4.1
Straw, surface-applied	2.7	1.8	5.9*	5.0
Straw, incorporated	2.8*	1.7	5.8*	4.8

* Significantly different from the control at the 5% level.

3.5.2 Effect of inorganic N and organic fertilizers on rice plant-associated N_2-fixation.

3.5.2.1 Effect of inorganic N on ARA associated with rice plants (Experiment 1)

Plant ARA of IR50 and IR54 were measured several times during the crop cycle at different levels of inorganic N fertilizer. Analysis of variance of ARA per plant and ARA per plant dry weight showed significant differences due to N treatment and growth stage. In IR54, only ARA expressed on a per plant basis was significantly different at different growth stages. On the other hand, total plant dry weights were significantly different in both varieties at all N levels (data not shown). At an early stage of plant growth (22-23 DAT), ARA of both varieties expressed on a per plant basis showed a slight inhibition specially in treatments with higher doses of N (Figs. 3.2 and 3.3). At maximum tillering and just before the second application of urea, ARAs of N-treated plants were always higher than in the control in both varieties. The responses of ARA to topdressing of N were different in both varieties; there were decreases in IR50 and increases in IR54. However, when only the fertilized treatments after the second application of N were compared, it was observed that the highest level of top-dressed N resulted in the lowest plant ARA in both varieties.

In this experiment, the inhibitory effect of N fertilizer on plant ARA was demonstrated by measurements made a few days after N application, but it was

also shown that this inhibitory effect could be overcome at later stages, and that the increased biomass due to N fertilizer could lead to a higher ARA per plant. The effect of inorganic N fertilizer on plant ARA is therefore dependent on the time interval between N application and ARA measurements and the response of the rice plant to fertilization in terms of dry weight.

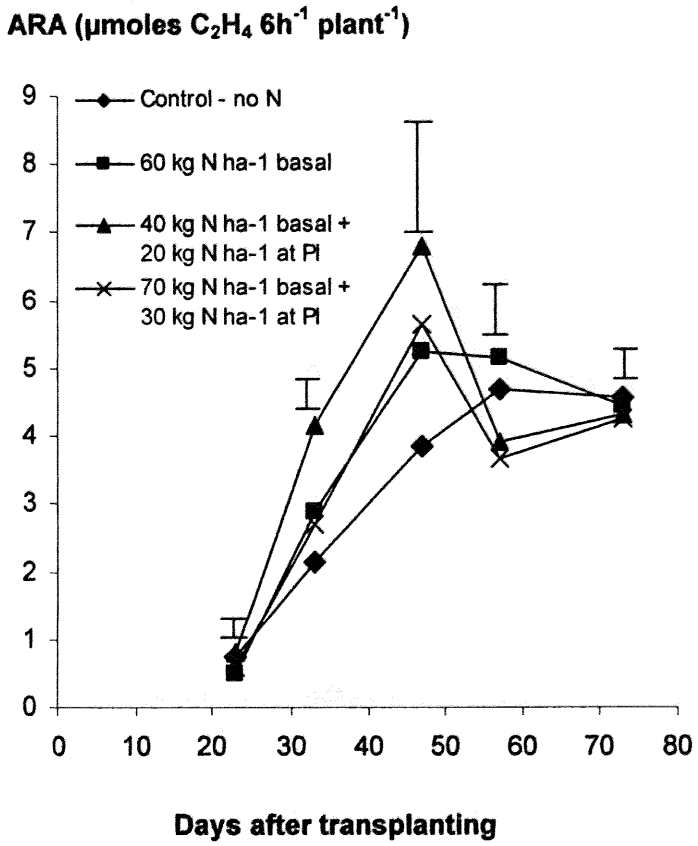


Fig. 3.2. Effect of different levels of inorganic N fertilizer on ARA associated with IR50. Vertical bars represent standard error of the mean. Basal application of urea was made 1 day before transplanting and top dressing was made at PI stage (47 days after transplanting) (Experiment 1).

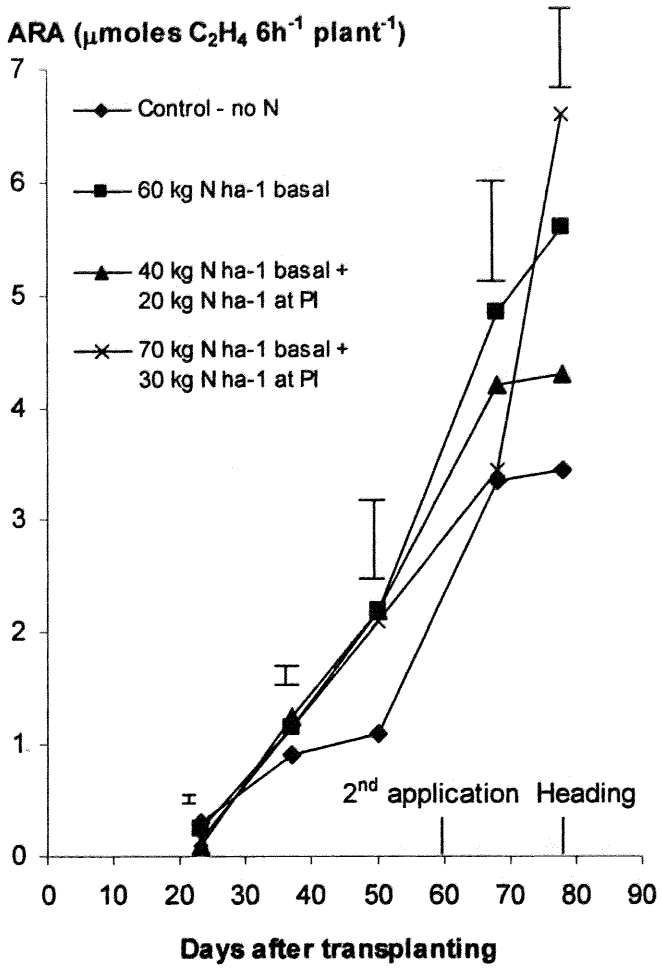


Fig. 3.3. Effect of different levels of inorganic N fertilizer (urea) on ARA associated with IR54. Basal application of urea was made 1 day before transplanting and top dressing was made at PI stage (60 days after transplanting) (Experiment 1).

3.5.2.2 Effect of incorporation of the preceding leguminous and non-leguminous GM crops on ARA associated with the rice plant (Experiments 2 and 3).

In experiments 2 and 3, the effects of incorporation of different legume and non-leguminous GM crops (grown and incorporated in the same field) on plant-associated ARA were compared with controls (no nitrogen) and with inorganic N fertilizer. In experiment 2, the control treatments included weed-free and weedy fallow with and without inorganic N. Measurements of ARA and plant dry weights were made for 3 consecutive days at the heading stage in experiment 2, and at both maximum tillering and heading stages in experiment 3. The amount of N incorporated in different GM treatments and their effects on ARA are shown in Tables 3.7 and 3.8.

Table 3.7. Effect of different leguminous green manure species on plant-associated ARA at heading stage of IR54 during the dry season (Experiment 2).

Treatment[a]	Amount of N ha^{-1} (as urea and/or GM crop) applied	ARA (nmol C_2H_4 6 h^{-1})[b]	
		Per plant	Per gram plant dry weight
Control			
Weed free	0	2100d	70 c
Weedy fallow	21	2100 d	75 bc
Weedy fallow + urea (70 kg N ha^{-1})	21+70	2300 cd	67 c
Incorporation of GM			
Crotolaria juncea	144	4700 a	87 ab
Vigna radiata	93	4700 a	97 ab
Sesbania acualeata	173	4000 ab	82 abc
S. aculeata + urea (35 kg N ha^{-1})	173+35	4200 a	82 abc
Dolichos lablab	76	3200 b	831 bc
Standard error		281	4.9

[a] Urea was applied in split: ½ basal and ½ at PI (55 days after transplanting). GM crop was grown for 60 days and incorporated. In weedy fallow treatments, weeds accumulated 21 kg N ha^{-1} in 60 days.

[b] In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test (DMRT).

Table 3.8. Effect of inorganic and GM N on plant-associated ARA of IR54 during the dry and wet seasons (Experiment 3).

Treatment[a]	Amount of N ha^{-1} (as urea and/or GM crop) applied[a]	ARA (nmol C_2H_4 6 h^{-1})			
		Per plant		Per g plant dry weight	
		Maximum tillering	Heading	Maximum tillering	Heading
Dry season					
Control	0	2400	4500	121	86
Urea-applied	50	2300	5300	99	87
Azolla-incorporated	98	2000	6300**	68*	99*
Sesbania-incorporated	65	2200	6500**	89*	91
Wet season					
Control	0	1000	3200	83	107
Urea-applied	50	1200	3500	68	95
Azolla-incorporated	86	1100	3600	79	67*
Sesbania-incorporated	78	1200	4100	83	88

[a] Urea was applied in split: ½ basal and ½ at 50 days after transplanting; *Azolla microphylla* was grown for 30 days and incorporated 3 times before transplanting and one time, 1 month after transplanting. Sesbania was grown for 52 and 45 days during the dry and wet seasons, repectively, and incorporated 1 day before transplanting.

*, ** significantly different from the control at the 5% and 1% level respectively.

Acetylene reducing activity expressed per plant or per gram plant dry weight basis were not significantly different from among control treatments – weed-free, weedy fallow, and weedy fallow plus 70 kg N ha^{-1}. However, ARAs per plant in

all GM treatments were significantly higher than in all three controls. Acetylene reducing activities expressed on a per gram plant dry weight basis were, on the other hand, significantly higher only in *Crotolaria juncea*- and mungbean (*Vigna radiata*)-incorporated treatments.

In experiment 3 significant effects were found more often in the DS than in the WS (Table 3.8). Acetylene reducing activities per plant were significantly higher at the heading stage in both (*Azolla* and *Sesbania*) GM treatments than in the control and urea treatments. Acetylene reducing activity per gram plant dry weight was only higher in the *Azolla*-incorporated treatment in the DS but the same treatment gave significantly lower activities
in the WS. Acetylene-reducing activities at the maximum tillering stage, on the other hand, were unaffected in both seasons except that ARA per gram plant dry weight decreased significantly in *Azolla* and *Sesbania*-incorporated treatments during the DS.

3.5.2.3 Effect of inorganic N, organic (FYM), and GM fertilizers on ARA associated with rice plants as affected by the preceding upland or fallow crop (Experiment 4).

Acetylene reducing activity per plant from treatments with inorganic N were significantly higher than that with no N or with FYM. On the other hand, ARA per plant dry weight was the same in all treatments except for the mungbean-incorporated treatment which gave a significantly higher ARA per plant dry weight than all the other treatments (Table 3.9). Whether leaving the land fallow

Table 3.9. Effect of preceeding upland or fallow crop on rice plant-associated ARA at the heading stage of IR54 during the WS (Experiment 4).

Cropping sequence	Amount of N ha^{-1} (as urea, FYM, or GM) applied[a]	ARA (nmol C_2H_4 6 h^{-1})[b]	
		Per plant	Per g plant dry weight
Fallow-Rice-Maize	20+0	1460 c	66 b
Fallow-Rice+FYM-Maize	20+103	1400 c	62 b
Fallow-Rice+urea-Maize	20+35	2000 a	69 b
Fallow-Rice+urea-Maize	20+70	1800 ab	57 b
Fallow-Rice+urea-Maize	20+105	2000 a	57 b
Sesbania aculeata-Rice-Maize	171	1800 a	59 b
Vigna radiata-Rice-Maize	100	1800 ab	82 a
Standard error		105.4	3.8

[a] Urea was applied in split: ½ basal and ½ at PI (55 DAT), Sesbania and Vigna crops were grown for 60 days and incorporated. In fallow treatment, weeds accumulated 20 kg N ha^{-1}.
[b] In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

or growing a GM crop before rice affects ARA is not clear. However, as in the other experiments, it seems that ARAs associated with plants grown after upland or fallow crop were lower than ARAs associated with plants grown under continuous wetland cropping.

3.5.2.4 Effect of long-term application of inorganic N, organic and GM fertilizer (Experiment 5).

The effect of different sources of inorganic N fertilizer, organic, and green manures keeping the amount of applied N constant was studied. Green manures incorporated in this experiment were obtained from another field. Acetylene-reducing activity was measured on plants of the 13th crop, grown continuously with the same treatments at 32, 47, and 70 DAT (Table 3.10).

Table 3.10. Effect of mineral N fertilizer, GM and organic manure on plant-associated ARA of IR64 during the DS (Experiment 5).

Treatment[a]	ARA (nmol C2H4 6 h^{-1})[b]					
	32 DAT		47DAT		70DAT	
	ARA per plant	ARA per g plant dry weight	ARA per plant	ARA per g plant dry weight	ARA per plant	ARA per g plant dry weight
Control	564 ab	142 a	1030 a	90 a	3280 a	102 ab
Prilled urea (best split)	317 c	53 b	830 a	41 bc	3817 a	85 ab
Urea supergranule	421 bc	82 b	603 a	32 c	3793 a	84 ab
Azolla incorporation	536 abc	129 a	1150 a	83 a	2872 a	75 ab
Sesbania rostrata incorporation	521 abc	124 a	860 a	78 a	3133 a	75 ab
Rice straw incorporation	625 ab	152 a	990 a	68 ab	2642 a	59 b
Rice straw compost incorporation	717 a	145 a	850 a	63 abc	3535 a	109 a
Standard error	71	13	141	10	498	14

[a] Urea, GM and organic manure were applied in amounts equivalent to 116 kg N ha^{-1}. Prilled urea was applied in split: 2/3 basal and 1/3 topdressed at 7 days before PI (40 DAT). The total amount (dry weight t ha^{-1}) of Azolla mycrophilla, Sesbania, straw, and straw compost were 8.5, 8.8, 28.0, and 22.0, respectively.

[b] In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Acetylene reducing activities per plant were significantly different among treatments at 32 DAT. Prilled urea-treated plants had significantly lower ARAs than the control plants. Acetylene reducing activities on a per plant dry weight basis among the treatments were, on the other hand, significantly different in all

three samplings. Both inorganic N fertilizer treatments inhibited ARA per plant dry weight significantly compared to the control at 32 and 47 DAT.

3.5.2.5 Effect of integrated use of inorganic N and organic (straw) fertilizer on ARA associated with rice plants (Experiment 6).

The purpose of this experiment was to study the effect of different time and method of application on organic material (straw) with high C:N ratio in combination with inorganic N fertilizer. Although straw application has been found to stimulate non-rhizospheric N_2 fixation (Section 2.5.3), N deficiency due to immobilization of N by straw has also been reported (Ponnamperuma, 1984). Therefore, both methods of straw application were combined with topdressing of N. For comparison, two controls (urea topdressing and urea basal with topdressing) were included. ARA was measured at 33 and 63 DAT and at the heading stage (90 DAT) (Table 3.11).

Table 3.11. Effect of two modes of straw application on plant-associated ARA of IR64 during the dry season.

Tretatment[a]	Amount of N ha^{-1} as urea or organic manure applied	ARA (nmol C2H4 6 h^{-1})[b]					
		33 DAT		63 DAT		90 DAT	
		ARA per plant	ARA per g plant dry weight	ARA per plant	ARA per g plant dry weight	ARA per plant	ARA per g plant dry weight
Control (0-30)	30	710	85	2700	80	5600	90
Urea (5-30)	80	860	79	3400	95*	6900	104
Chopped straw applied before transplanting + urea (0-30)	60	910*	101*	4000	124*	5900	92
Long straw applied after transplanting + urea (0-30)	60	830	94	2900	90	7500*	104
Standard error		64	5	238	5	504	6

[a] Chopped straw (5 t ha^{-1}) was surface-applied 21 days and incorporated 2 days before transplanting; long straw (5 t ha^{-1}) was surface-applied 25 days after transplanting. All treatments received a top dressing of 30 kg N ha-1 at the PI stage. Urea treatment also received basal application of 50 kg N ha^{-1}.

* Significantly different from the control at the 5% level.

In general, rice plant ARA was higher in plots where straw and split urea were applied than in the control. Straw application before transplanting significantly increased ARA per plant and per plant dry weight at 33 and 63 DAT. Straw

application after transplanting significantly increased only ARA per plant at heading.

The higher ARA at an early stage of plant growth due to straw application before rice transplanting might have coincided with the release of nutrients from straw decomposition as the half life of straw was reported to be about 43 days (Neue, 1985). This observation is further confirmed by higher ARA at the heading stage due to straw application after transplanting (25 DAT) (Table 3.11). Such results suggest the possibility of split application of straw to increase associative N_2-fixation.

Table 3.12. Summary of experimental results on the effect of inorganic N and organic fertilizers on rice plant-associated ARA at heading stage.

Treatment Category	No. of trials	N applied (kg ha^{-1})		% increase or decrease over control					
		Mean	SE	ARA per plant		ARA per g plant dry weight		Plant dry weight	
				Mean	SE[a]	Mean	SE	Mean	SE
Grain legume GM	3	90.0	7.1	64.0	29.8	26.6	5.9	30.2	17.1
Non-grain legume GM	8	129.3	15.2	50.8	16.5	-2.6	7.6	45.7	8.7
Urea	17	77.0	8.0	28.0	6.8	-8.8	2.8	37.8	7.7
Non-legume GM	3	100.0	7.0	12.2	14.6	-16.4	16.1	41.6	16.6
Rice straw and FYM	5	83.0	18.0	4.4	8.6	-4.6	10.0	15.4	8.1

[a] SE, standard error

Table 3.12 summarizes the effects of inorganic and organic fertilizer on plant ARA and dry weight at heading stage. Data for the heading stage alone were considered because the ARA was found to be highest at this stage. Based on the nature of fertilizer used, the treatments were divided into the following categories: (1) grain legume GM (e.g. *V. radiata* (mungbean) and *D. lablab* (lablab); (2) non-grain legume GM (e.g. *Sesbania* and *Crotolaria*); (3) inorganic N fertilizer (urea); (4) non-legume GM (*Azolla*); (5) rice straw, and FYM. The average amounts of N applied in the different fertilizer categories and the percentage increase or decrease of ARA and plant dry weight over their respective controls were determined. Acetylene-reducing activity per plant increased in all the treatments except in the FYM treatment, which shows a slight decline (3.3%). Acetylene-reducing activity per plant dry weight was lower in all treatments except in the grain legume GM treatments which showed an average increase of 27%. On the other hand, the plant dry weight increased in all categories of fertilizer treatments.

3.6 Discussion

3.6.1 Effects of 2 modes of straw application to a wetland rice field on acetylene-reducing activity (ARA) associated with the rice plant and non-rhizospheric soil, and on the N_2-fixing bacterial populations.

Results from this study show that the application of straw to the submerged paddy soil can support appreciable N_2-fixation (C_2H_2 reduction) and multiplication of various bacteria. The occurrence of the ARA peak during the initial period of straw application is in agreement with the decomposition pattern of straw (Neue, 1985). Neue (1985) found 50% of ^{14}C remaining after 43 days that labeled straw was incorporated (5 t ha^{-1}) in a submerged rice field at the IRRI farm with similar cultural, environmental, and edaphic conditions used in the present experiment. A similar decomposition pattern of straw was also found by Watanabe (1984). Matsuguchi (1979), on the other hand, reported that in the flooded rice soils of Japan, the onset of the stimulatory effect of straw incorporation on ARA came at about 4 weeks and it persisted for about 10 weeks. The delay could be due to the slow decomposition of straw under temperate conditions.

The reason for the significant increase in plant associated ARA of IR42 attributed to straw application cannot be ascertained. The degrading straw may have directly supplied the substrate for the rhizospheric N_2-fixing microflora or the rice plant may have absorbed more nutrients from the degrading straw and supported higher N_2 fixation in return. The high incidence of total and N_2-fixing heterotrophs in the soil in association with degrading straw pieces are in agreement with the higher ARA.

Long-term straw application has been shown to maintain higher organic matter and N content of the soil (Ponnmperuma 1984; Gotoh et al., 1984). The anaerobic conditions in wetland rice fields stimulate the formation and accumulation of intermediate C substrates due to the retarded organic matter decomposition which not only favors N conservation and fixation but also the immobilization of N. Although the contribution of straw N and straw-associated, biologically-fixed N to the current N needs of rice may not be great, the long-term effects are substantial.

It may be concluded that the decomposition of straw especially with surface application provides energy for growth and N_2-fixation by heterotrophs. Acetylene reduction is a short-term indirect method and can only be used as an index for measuring the difference in N_2-fixation among treatments. Thus, ARA results should

be substantiated by nitrogen balance data. Most of the straw and fixed N does not seem to be available to the plant for grain production and probably become immobilized to the soil organic N pools. A better knowledge of the kinetics of biomass N and improved cultural and management methods for its utilization by the rice plant is needed.

3.6.2 Effect of inorganic N and organic fertilizers on rice plant-associated N_2-fixation.

Inorganic and organic fertilizers may exert different effects on N_2 fixation associated with rice. One of these could be the inhibitory effects due to the combined form of N (as in the case of inorganic fertilizer) or to toxic compounds released by the decomposition of organic materials. The former may be more important than the latter. Such inhibitory effects, however, may not be very important and long lasting, especially in tropical rice fields, where there is intense biological activity. Furthermore, the inorganic N disappears in flooded soil systems after 30-50 days of application of fertilizer (Watanabe and Inubushi, 1986; Nagarajah, 1987). The second type of effect may be a direct or indirect stimulatory effect. The direct effect could be due to the release of carbon substrates and other nutrients from the organic fertilizers, and the indirect effect could be increased plant growth and greater release of carbon compounds by the plant.

Infrequent and insignificant inhibition of ARA at the early growth stages, and frequent and significant stimulation of ARA at the later stages due to different fertilizers were found. However, the stimulation in ARA per gram plant dry weight was not as much as that in ARA per plant; in fact, there was some decline in several cases, compared to the control. Interestingly, there were also no correlations between the increases or decreases in ARA per plant or ARA per gram plant dry weight and the amount of N applied (Figs. 3.4 and 3.5). This rules out the possibility that the inhibition or stimulation of ARA is a monotonic function of the amount of N. The relationship between percent increase or decrease of ARA per plant or ARA per gram plant dry weight and percent increase or decrease of total plant dry weight were also examined (Figs. 3.6 and 3.7). There was a correlation between ARA per plant and plant dry weight, but there was no correlation between ARA per plant dry weight and plant dry weight. The absence of correlation in the latter case is due to the fact that the increase in plant dry weight is much more than the increase in the specific ARA (ARA per plant dry weight).

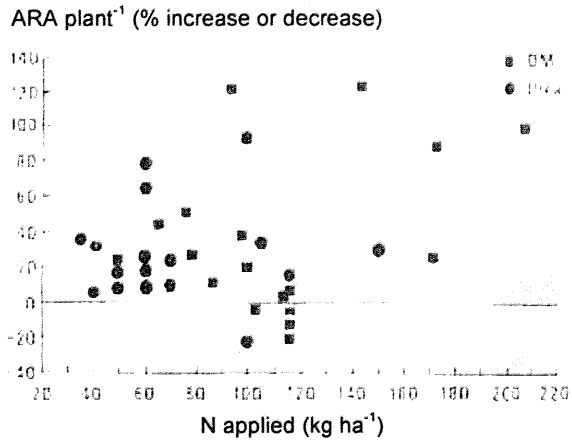


Fig. 3.4 Relationship between % increase in ARA per plant at heading and the amount of N (kg ha^{-1}) as urea and organic fertilizers applied.

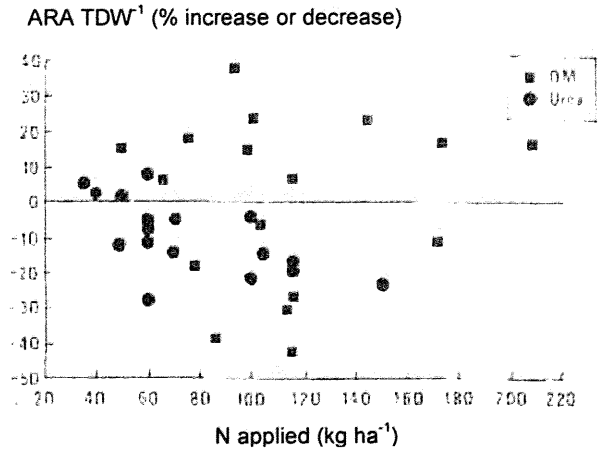


Fig. 3.5 Relationship between % increase or decrease in ARA per gram plant dry weight at heading and the amount of N (kg ha^{-1}) as urea and organic fertilizers applied.

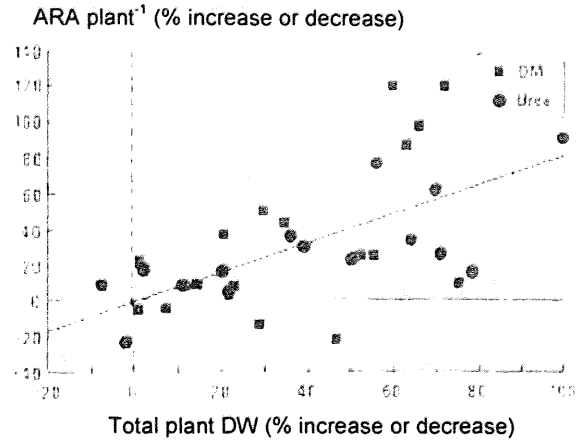


Fig. 3.6 Relationship between % increase or decrease in ARA per plant and % increase or decrease in total plant dry weight at heading stage.

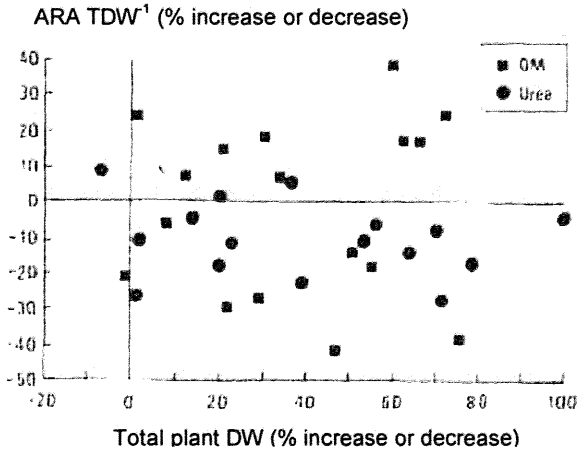


Fig. 3.7 Relationship between % increase or decrease in ARA per plant dry weight and % increase or decrease in total plant dry weight at heading stage.

The significant increases in specific N_2-fixing activity and the total plant dry weight at heading stage in mungbean- and *Crotolaria*-incorporated treatments (Tables 3.13 and 3.14) are interesting and noteworthy. It may be worthwhile to investigate whether the rhizobia, which are being inoculated in large numbers through the incorporation of its host biomass, are making an active N_2-fixing association with the rice plant. Rhizobia of some legumes such as Sesbania rostrata used as GM in rice has been reported to be fixing N_2 ex planta (Dreyfus et al, 1983).

Table 3.13. Effect of different leguminous green manure species on plant-associated ARA at heading stage of IR54 during the dry season (Experiment 2).

Treatment[a]	Amount of N ha^{-1} (as urea and/or GM crop) applied	ARA (nmol C_2H_4 6 h^{-1})[b]	
		Per plant	Per gram plant dry weight
Control			
Weed free	0	2100d	70 c
Weedy fallow	21	2100 d	75 bc
Weedy fallow + urea (70 kg N ha^{-1})	21+70	2300 cd	67 c
Incorporation of GM			
Crotolaria juncea	144	4700 a	87 ab
Vigna radiata	93	4700 a	97 ab
Sesbania acualeata	173	4000 ab	82 abc
S. aculeata + urea (35 kg N ha^{-1})	173+35	4200 a	82 abc
Dolichos lablab	76	3200 b	831 bc
Standard error		281	4.9

[a] Urea was applied in split: ½ basal and ½ at PI (55 days after transplanting). GM crop was grown for 60 days and incorporated. In weedy fallow treatments, weeds accumulated 21 kg N ha^{-1} in 60 days.

[b] In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test (DMRT).

Table 3.14. Effect of preceeding upland or fallow crop on rice plant-associated ARA at the heading stage of IR54 during the WS (Experiment 4).

Cropping sequence	Amount of N ha^{-1} (as urea, FYM, or GM) applied[a]	ARA (nmol C_2H_4 6 h^{-1})[b]	
		Per plant	Per g plant dry weight
Fallow-Rice-Maize	20+0	1460 c	66 b
Fallow-Rice+FYM-Maize	20+103	1400 c	62 b
Fallow-Rice+urea-Maize	20+35	2000 a	69 b
Fallow-Rice+urea-Maize	20+70	1800 ab	57 b
Fallow-Rice+urea-Maize	20+105	2000 a	57 b
Sesbania aculeata-Rice-Maize	171	1800 a	59 b
Vigna radiata-Rice-Maize	100	1800 ab	82 a
Standard error		105.4	3.8

[a] Urea was applied in split: ½ basal and ½ at PI (55 DAT), Sesbania and Vigna crops were grown for 60 days and incorporated. In fallow treatment, weeds accumulated 20 kg N ha^{-1}.

[b] In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

It may be concluded that the application of inorganic N and different organic (including GM) fertilizers does not inhibit N_2-fixation associated with the rice plant. N_2-fixation on a per plant or unit area basis is either enhanced or not affected but the effect is generally proportional to the plant biomass. The enhancement of N_2-fixation in proportion to the plant biomass by incorporating some leguminous plants is interesting and worth investigating.

CHAPTER 4

GENOTYPIC SELECTION FOR IMPROVED N USE EFFICIENCY IN RICE

4.1 Summary

Field screening trials were conducted in two dry seasons to assess the variability in grain yield, N uptake and N utilization of 180 rice genotypes, mostly lowland indica improved varieties or elite breeding lines of different growth durations without the addition of N fertilizer and to identify genotypes with the potential to produce high yields at suboptimal N levels through efficient uptake and/or utilization of N.

Available soil N was lower in year 2 (20 kg ha^{-1}) than in year 1 (64 kg ha^{-1}). In both years, significant differences in grain yield, N uptake and N utilization efficiency (NUE) were observed among genotypes within each growth duration group. Genotypes varied in their response to change in available soil N. The average increase in grain yield for each kilogram increase in N uptake was 61.9 kg in year 1 and 82.7 kg in year 2. However, some genotypes absorbed similar amounts of N but produced different grain yields and/or total dry matter.

Some genotypes with similar harvest index (HI) exhibited significantly different NUEs. Those with higher NUE had lower percentage straw N at maturity. Grain N concentration also affected NUE, but the coefficient of variation in percentage grain N among genotypes was less than that of percentage straw N.

The relative performance of genotypes in terms of NUE was more consistent than plant N uptake, based on rank correlations between the two trials. High N uptake and NUE were observed in IR131429-150-3-2-1-2 (NUE 65.4, N uptake 9.1 g m^{-2}) in the early-duration group, IR44 (NUE 67.2, N uptake 8.3 g m-2) in the medium duration group, and IR39323-182-2-3-3-2 (NUE 64.8, N uptake 9.3 g m-2) in the late-duration group. The study identified genotypes which may possess promising traits for improved N uptake and utilization efficiency.

4.2 Introduction

Rice derives more than half of its N requirement from the soil N pool in both low- and high-input production systems (Koyama, 1971; Partick and Reddy, 1976; Kundu and Ladha, 1995). Long-term N balance experiments have shown that regardless of the amount of N removed by rice, the soil N content is maintained through biological N_2-fixation by associative and free-living microorganisms (Koyama and App, 1979; App et

al., 1984). Rice genotypes differ in their ability to stimulate BNF (as discussed in chapter 2), and also in soil N uptake and in the efficiency of N utilization (App et al, 1986; Broadbent et al., 1987; De Datta and Broadbent, 1990). This raises the possibility of exploiting rice genotypes that absorb large amounts of N and produce high yields, and /or those that produce higher yields from a given amount of N absorbed. Much has been done to improve N utilization through agronomic management e.g., the timing, rate, placement, and source of fertilizer (De Datta and Patrick, 1986; Fillery and Vlek, 1986; De Datta and Buresh, 1989; Schnier et al., 1990; Cassman et al., 1994; Shoji and Kano, 1994; Kundu and Ladha, 1995; Singh et al., 1995; Peng et al, 1996; Balasubramanian et al., 1999; Mohanty et al, 1999). However, little effort has been made to explore the potential genotypic variability in N uptake and utilization efficiency. Having N-efficient genotypes will result in lower input requirements, reduced production costs and reduced pollution and environmental problems.

4.3 Objectives

The objectives of this study were:

a. To assess the extent of genotypic variability in grain yield in relation to N uptake and utilization and related plant parameters;
b. To identify genotypes with potential to produce high yield at suboptimal N levels through efficient uptake and utilization of N;

4.4 Materials and Methods

4.4.1. Experimental site

The screening trials were conducted at the IRRI wetland experimental farm. The soil had a bulk density of 0.885 g cm^{-3}; total N, 0.12%; total C, 1.10%; available Olsen P, 26.1 ppm; CEC, 31 meq 100 g^{-1}; and pH (1:1) H2O, 6.2. The experiments were conducted under similar conditions for 2 consecutive dry seasons but with a significantly lower available soil N content during the second year. Exchangeable ammonium N concentration was 36.3 mg kg^{-1} during the first year and 11.2 mg kg^{-1} during the second year. A crop of maize grown between the trials lowered the soil NH_4^+-N. Three consecutive crops of rice were grown without N fertilizer prior to the actual screening to reduce soil NH_4^+-N and homogenize the fields. Measurements of plow depth, NH_4^+-N and grain yield from different sampling areas within a block were made to ensure uniformity. Fields were plowed and harrowed three times with a

hand tractor, correcting for some differences in depth of the plow layer. Phosphorus and potassium were incorporated during the last harrowing at the rate of 60 kg ha^{-1} each of P_2O_5 and K_2O.

4.4.2 Soil sampling and analyses

Each field was divided into 4 blocks, 24 x 36 m each and further subdivided into15 plots for soil sampling. Composite samples were prepared for each plot from three random borings 4 cm in diameter and 20 cm deep. The total weight of each composite sample and its moisture content were determined to obtain the bulk density. Total N was determined by the Kjekdahl method of Bremner (1960) and NH_4^+-N by the method of Bremner and Keeney (1966). The apparent plow depth was measured for every 4-m^2 area within a block by applying manual pressure on a meter stick until it reached the hard pan. Measurements were done by only one person so that approximately equal pressure was applied each time.

4.4.3 Description of genotypes and experimental design

One hundred eighty genotypes of different growth durations [60 each of early (100-110 days, medium (111-119 days) and late (120-135 days)], diverse tillering capacities, plant heights and genetic/geographic backgrounds were tested (Table 4.1). Forty-six of the genotypes were non-IRRI varieties/lines; among them, 12 originated from Sri Lanka, 11 from India, 4 from Indonesia, 3 each from Philippines and Bangladesh, 2 each from China, Vietnam and Korea, and 1 each from Zanzibar, Colombia, Taiwan, Cuba, Nepal, Liberia, and Cote d'Ivoire. All except seven genotypes were lowland indica improved varieties or elite breeding lines. Of these seven genotypes, five were traditional and two were upland varieties. Since part of this study aims to develop breeding lines with low and high abilities for soil N uptake, and utilization efficiency, there was a need to identify parents possessing the desired traits among genotypes of diverse characteristics including traditional varieties and those which may be considered inferior. The group-balanced block design (Gomez and Gomez, 1984) was employed with three groups based on growth duration and four replicates. Seeds were sown singly in 1-cm^3 sections of seedling trays previously filled with wet soil. After 14 days, the seedling were pulled one by one with the soil an roots intact and transplanted in the field at 20x20 cm spacing. Eighty-eight hisll

(11 x 8 rows) of each genotype were transplanted in each block. Water level was maintained at about 15-20 cm above the soil surface and pesticides were applied

Table 4.1a Early-duration genotypes[a] used and their characteristics[b]

No.	Variety / Line	GD	TILNO		PLH	
			1	2	1	2
1	IR50	106	17	13	78	64
2	IR50376-80-1-1-2-2	109	12	11	69	59
3	IR25924-92-1-3	105	14	13	83	74
4	PSBRc10	106	14	10	72	63
5	IR51009-155-2-3-3	107	13	11	79	64
6	IR41996-50-2-1-3	102	11	12	81	67
7	IR50351-2-41-3-3-2-3	108	13	11	74	65
8	IR51009-62-3-3-3	109	12	15	69	66
9	IR52256-190-2-2-1	109	14	14	75	63
10	IR52256-190-2-2-1	104	13	12	67	59
11	IR52341-60-1-2-1	106	10	8	75	68
12	IR50358-102-2-3-3	110	14	13	79	66
13	IR52280-179-2-1-2	108	12	9	66	63
14	IR42068-22-3-3-1-3	106	12	11	76	67
15	IR42000-211-1-2-2-3	105	12	10	83	66
16	IR50363-61-1-2-2	109	13	11	82	64
17	IR51008-89-2-1-2	109	11	9	79	66
18	IR8566-94-1-2-5	110	12	10	65	61
19	IR52287-153-1-1-2	109	11	11	81	64
20	IR45138-115-1-1-2-2	109	12	11	78	64
21	IR48613-54-3-3-1	109	11	10	74	63
22	IR49460-54-2-2-2-3	108	15	12	85	70
23	IR49517-23-2-2-3-3	106	12	10	85	74
24	Ma-wei-chan[l]	101	10	11	135	102
25	IR8455-78-1-3-3	107	13	10	81	69
26	IR8608-167-1-2	103	14	13	77	66
27	IR8866-30-3-1-4-2	108	14	11	90	73
28	IR62	107	15	11	77	69
29	IR54791-B-B-1	109	13	11	86	69
30	IR54819-B-B-1	109	12	11	80	69
31	IR54896-B-B-233	104	12	9	89	77
32	IR30	106	12	10	84	69
33	IR10198-66-2	102	11	14	86	76
34	IR54742-11-10-13-21-2	110	15	11	83	70
35	IR13240-82-2-3-2-3-1	109	14	11	71	62
36	IR13240-56-3-2-1	109	11	11	83	70
37	IR29692-34-1-3-2-2	109	12	10	81	61
38	IR31851-96-2-3-2-1	109	13	11	78	66
39	IR13429-150-3-2-1-2	110	14	13	78	65
40	25924-51-2-3	104	16	14	69	61
41	C662083	109	10	10	78	71
42	IR36	107	16	12	75	64
43	IR29725-117-2-3-3	109	13	9	80	65
44	IR31802-48-2-2-2	109	12	11	82	69
45	Milyang 5 (Gayabyeo)	104	12	12	72	67
46	UPR 231-28-1-2[U]	99	13	11	71	62
47	IR32876-54-2-2-2	110	16	12	77	70
48	RNR-1429	106	12	9	90	78
49	IR64	110	11	11	78	67
50	BG1203	103	13	11	83	68
51	BG367-4	107	12	9	88	73
52	IR44530-41-1-2-1	110	11	10	81	70
53	Sokotera[l]	105	10	12	115	93
54	IR45138-131-2-1-1-3	109	10	8	87	74
55	Hua-chou-chi-mo-mor[l]	109	11	10	111	91
56	IR21912-131-2-1-1-3	109	13	13	80	63
57	IR65	108	13	12	75	64
58	IR52	103	18	13	76	65
59	IRAT110[u]	109	10	7	89	73
60	IR5492-B-B-250	106	15	11	72	64

[a] [l] and [u], traditional and upland varieties.
[b] GD, average growth duration observed for the 1st and 2nd year; TILNO and PLH, tiller number (per plant) and plant height (cm) at flowering for the 1st and 2nd year.

Table 4.1b Medium-duration genotypes[a] used and their characteristics[b]

No.	Variety / Line	GD	TILNO		PLH	
			1	2	1	2
61	IR20	113	12	9	80	74
62	IR54751-2-44-15-2-2	111	14	9	76	68
63	IR50350-101-3-2-1-2	113	16	11	77	64
64	IR50391-100-2-3-3-2	112	11	10	63	57
65	IR51011-16-2-2-3	111	11	10	82	67
66	M1-28	115	8	6	109	94
67	IR54	116	12	12	84	72
68	CICA 8	119	13	11	84	75
69	Suweon 290	115	10	8	85	75
70	IR54819-B-B-172	113	12	10	84	74
71	IR54819-B-B-226	113	13	8	80	71
72	IR54819-B-B-232	112	9	8	84	78
73	IR54819-B-B-278	112	12	10	75	68
74	IR54819-B-B-396	116	9	10	78	78
75	IR54896-B-B-139	113	11	9	95	75
76	BG34-8	113	13	9	80	65
77	BG94-1	112	11	11	78	70
78	BG380-2	116	10	9	87	78
79	IR22082-41-2	119	12	11	81	72
80	IR24632-206-3-2-2	116	10	11	77	70
81	IR35366-28-3-1-2-2	116	12	13	82	78
82	IR49457-33-1-2-2-2	114	12	9	80	68
83	IR46997-69-2-2-2	112	11	10	75	64
84	IR50990-121-3-3-1	117	13	12	83	72
85	IR47761-27-1-3-6	111	12	11	92	82
86	IR45067-136-1-1-1-2	117	11	10	86	84
87	IR54742-31-9-26-15-2	115	10	8	84	81
88	Pinulot 330	115	10	9	126	106
89	IR44 (IR2863-38-1-2)	117	15	12	88	76
90	IR28228-119-2-3-1-1	117	13	9	84	76
91	C712039	112	10	10	86	67
92	IR34	116	12	9	92	82
93	IR45	116	15	11	82	66
94	IR9884-54-3-1E-P1	117	13	12	89	75
95	BG90-2	116	12	12	88	75
96	IET1444	111	12	10	90	74
97	IR4563-52-1-3-6	115	14	13	84	71
98	IR37839-101-1-11R	114	10	8	89	83
99	IR54790-B-B-38	112	10	9	89	71
100	IR58125-B-B-42	119	9	8	91	83
101	IR66072-18-1-2	119	7	6	101	91
102	M28D-159-4	112	12	10	73	61
103	IR21734-16-3-2-2-2	119	11	9	94	76
104	IR31805-20-1-3-3	113	15	13	79	63
105	IR21820-154-3-2-2-3	119	12	11	89	80
106	MTU5410-14	115	11	10	90	80
107	BR11	115	11	10	89	77
108	IR25604-99-1-3-2-2	117	9	9	81	72
109	BW295-5	115	10	10	87	80
110	ECIA66-129-1-1-1	118	10	7	98	87
111	B4414F-MR-6-1	114	9	9	94	80
112	X.3-D.T	117	10	9	88	77
113	IR13146-45-2	117	13	10	88	75
114	IR27316-96-3-2-2	117	12	9	84	71
115	IR25587-133-3-2-2-2	115	10	8	85	73
116	IR54742-6-20-3-22-2	115	10	9	94	82
117	Pelita I-1	115	9	8	102	77
118	IR54791-B-B-87	111	11	11	79	66
119	H-4	117	8	9	133	104
120	IR9764-45-2-2	116	12	10	82	74

[a] [f] and [u], traditional and upland varieties.

[b] GD, average growth duration observed for the 1st and 2nd year; TILNO and PLH, tiller number (per plant) and plant height (cm) at flowering for the 1st and 2nd year

Table 4.1c Late-duration genotypes[a] used and their characteristics[b]

No.	Variety / Line	GD	TILNO		PLH	
			1	2	1	2
121	IR8192-200-3-3-1-1	122	13	11	90	82
122	BR51-46-1-C1	121	11	11	86	78
123	IR54896-B-B-122	126	10	10	88	76
124	IR28154-101-3-2	124	9	8	93	85
125	BR51-91-6	124	10	9	110	97
126	KAU1727	121	12	8	86	85
127	IR49503-272-2-2-3-1-3	123	9	9	84	82
128	IR54742-1-11-17-26-1	122	13	8	82	73
129	IR51673-50-2-1	122	11	10	88	74
130	IR39485-151-2-1-3	124	12	9	99	82
131	IR48648-56-3-2-2-2	124	13	9	92	81
132	IR51672-186-1-1	128	11	12	88	74
133	IR49461-113-3-2-3	125	11	10	90	79
134	Palawan[l]	123	5	6	115	110
135	PR106	122	10	9	81	80
136	IR40	131	12	9	98	82
137	IR54752B	125	9	9	97	92
138	IR32	130	14	12	83	73
139	I438	120	14	11	85	77
140	IR48	127	10	78	96	85
141	BW100	120	10	10	109	91
142	IR31432-7-2	132	11	9	80	73
143	IR4595-4-1-13	124	10	11	97	92
144	IR40750-82-2-2-3R	120	12	9	86	77
145	IR66072-11-4-2	122	6	6	92	85
146	IR66072-11-8-6	123	7	4	96	83
147	IR8098-41-3	122	11	10	93	81
148	IR1552	122	9	9	86	82
149	DR33	120	14	10	82	77
150	IR5	132	12	11	103	88
151	IR42	127	12	12	88	76
152	IR70	126	12	12	84	78
153	DR32	120	11	9	83	78
154	IR37721-130-2-2-3-1	128	11	12	87	76
155	IR28118-138-2-3	124	10	9	97	84
156	IR29723-88-2-3-3	127	11	9	85	76
157	IR21848-65-3-2-2	130	12	11	95	84
158	IR33383-23-3-3-3	121	14	11	96	82
159	OR142-99	125	10	10	101	92
160	BR51-74-6/J1	123	10	8	100	90
161	IR28150-84-3-3-2	121	11	11	77	72
162	IR39323-182-2-3-3-2	122	13	12	81	72
163	IR27325-63-2-2	135	13	11	93	85
164	IR35346-28-3-3-1	129	11	10	85	76
165	IR19672-140-2-3-2-2	127	12	11	89	78
166	IR45131-45-2-2-1-3	132	11	10	93	80
167	IR45131-147-2-2-1-1	123	11	8	96	78
168	IR29337-36-3	124	12	10	128	117
169	Oking Seroni[l]	128	8	9	162	126
170	Pankaj	123	10	10	92	83
171	IR54891-B-B-134	122	10	11	87	81
172	IR54853-B-B-318	122	11	9	87	84
173	IR54853-B-B-324	130	10	10	97	85
174	KK15-23-C	129	11	9	124	114
175	BG11-11	120	13	10	93	77
176	BG400-1	120	11	9	96	81
177	Suakoko-8	128	10	10	114	98
178	Bhavani	121	8	8	100	100
179	IR29723-143-3-2-1R	129	12	10	90	85
180	IR54742-22-19-3R	127	10	8	105	98

[a] [l] and [u], traditional and upland varieties.

[b] GD, average growth duration observed for the 1st and 2nd year; TILNO and PLH, tiller number (per plant) and plant height (cm) at flowering for the 1st and 2nd year.

whenever necessary for plant protection.

4.4.4 Plant sampling and measurement of growth and yield parameters.

At maximum tillering, flowering (50% of the plant population) and maturity, five hills of each genotype per block were sampled by cutting the plant just above the ground. The five hills were taken from one row, avoiding unhealthy plants and those with missing neighbors. Plant height, tiller number and panicle number were determined for each of the five hills. The five hills were then pooled for measurements of yield, yield components and N uptake. In most of the genotypes, plants were harvested 30 days after flowering unless more than 10% of the grains were still green (in which case, they were allowed to ripen fully).

The grains were threshed manually and sun-dried. Filled and unfilled grains were separated using a seed blower. A seed counter (Count-A-Pak model 77) was used to count 1000 filled grains and all the unfilled grains. The weight of 1000 filled grains and total filled grain weight from five hills were determined, and immediately after, moisture content of the grains was measured using a Satake moisture meter model HM3A. Total filled grain dry weight and grain yield at 14% moisture were calculated. The dry weight of the unfilled grains was determined after oven drying to constant weight at 70°C. The straw was also oven-dried to constant weight at 70°C.

After the straw samples were weighed, they were chopped using a forage cutter, mixed and subsampled. The subsamples were ground (powder fine) using a Heiko vibrating sample mill and analyzed for total N in a Perkin Elmer 2400 CHN analyzer (Jimenez and Ladha, 1993). Similarly, the grains were subsampled, ground and analyzed for total N.

The parameters that were evaluated are the following:

1. Plant height in cm (PLH) = distance from the ground to the tallest leaf at flowering
2. Tiller number per plant at flowering (TILNO)
3. Grain yield in g m^{-2} (GY) calculated at 14% moisture = filled grain weight of 5 plants x 5 x (100-%moisture)/86
4. Total shoot dry matter in g m^{-2} (TDM) = (straw dry weight + total grain weight) of 5 plants x 5
5. Harvest Index (HI) = filled grain dry weight in g m^{-2}/TDM

6. 1000 filled grain dry weight in g (GW)
7. Number of filled grains m^{-2} (FG) = filled grain dry weight in g m^{-2} x 1000/GW
8. Number of unfilled grains in m^{-2} (UG) = total number of unfilled grains of 5 plants x 5
9. Total spikelet number m^{-2} (SPNO) = FG + UG
10. Percentage filled spikelets (%FSP) = FG/SPNO
11. Panicle number m^{-2} (PANO) = panicle number per plant x 25
12. Number of filled spikelets per panicle (FSPP) = FG/PANO
13. Percentage straw nitrogen at maturity (%SN)
14. Straw nitrogen in g m^{-2} (SN) = straw dry weight g m^{-2} x %SN/100
15. Percentage grain nitrogen (%GN)
16. Grain N in g m^{-2} (GN) = filled grain dry weight in g m^{-2} x %GN/100
17. Total plant N in g m^{-2} (TPN) = SN + GN
18. Nitrogen use efficiency (NUE) =filled grain dry weight in g m^{-2}/TPN

4.4.5 Statistical methods

Combined analysis of variance (Gomez and Gomez, 1984) was used to show the variation among genotypes in the two trials and also the variation between the two trials. This was done separately for each growth-duration group. Mean comparison was done by the Duncan's multiple range test. The IRRISTAT program, version 93-1 (Biometrics Unit, IRRI) was used for analyzing the data.

Principal component analysis (PCA, Jackson, 1991) was performed independently for each growth duration group on the correlation matrix of eight varieties – GY, HI, NUE, and TPN from two trials to obtain a single variable or principal component which may express the overall variability among genotypes in these parameters. The PRINCOMP procedures for PCA (Statistical Analysis System) was used.

Simple linear correlation analysis (Gomez and Gomez, 1984) was performed to estimate the linear response in grain yield to soil N uptake and its significance.

4.5 Results

4.5.1 Genotype variability in plant parameters as influenced by change in soil NH_4^+-N between years

Growth duration did not vary much between year 1 and year 2 in the early- and medium-duration genotypes with an average absolute difference and standard

deviation of 1.5±1.3 days in the early an 1.8±1.2 days in the medium. Among the late duration genotypes, growth duration was generally longer in year 2 than in year 1 with an average absolute difference and standard deviation of 5.3±3.4 days.

Growth duration had higher positive correlations with TDM, GY, and FSPP among the parameters measured and negative correlations with HI and %SN in both years. Percent GN also had a significant negative correlation with growth duration in year 1 but not in year 2. The correlation coefficient between growth duration and TPN was higher in year 2 than in year 1, but for NUE, the correlation coefficient was significant ($P<0.05$) in year 1 but not in year 2 (Table 4.2).

Table 4.2 Correlation of selected plant parameters with growth duration in year 1 and year 2.

Parameter	Correlation coefficient[a]	
	Year 1	Year 2
GY	0.331	0.354
TDM	0.617	0.647
TPN	0.233	0.461
NUE	0.232	0.087
HI	-0.401	-0.503
%GN	-0.409	-0.072
%SN	-0.424	-0.601
SPNO	0.171	0.304
%FSP	0.042	-0.206
GW	0.175	0.093
PANO	-0.245	-0.042
FSPP	0.457	0.356

[a] Significant at the 5% level if ≥ 0.146 and 1% level if ≥ 0.191.

Combined ANOV of 2 –year data showed significant differences among genotypes in all the parameters measured and between years in all except %SN in the early- and medium-duration group, FSPP in the early-duration group and %FSPP in the late-duration group. A significant interaction between genotype and year was also obtained for most of the parameters (Table 4.3).

Reduced soil N in year 2 resulted in lower PLH, TILNO (Table 4.1) GY, TDM, SPNO, PANO, FSPP, %GN, SN, GN and TPN but higher HI and NUE as compared with year 1 (Table 4.3). Parameters that were not appreciably affected by the change in soil N were %FSP, GW and %SN (Table 4.3). Based on rank correlations between years 1 and 2 data, GW, PLH, PANO, and FSPP appear to be the more stable parameters with correlation coefficients (r) greater than 0.6 in all growth duration

Table 4.3 Genotypic variability in plant parameters in year 1 and year 2.

Parameter	Range		Mean		Combined ANOV-F			CV
	Year 1	Year 2	Year 1	Year 2	Y	V	YxV	(%)
Early								
GY (g m^{-2})	332-698	180-505	514	395	**	**	ns	19.6
TDM (g m^{-2})	705-1147	385-791	899	634	**	**	ns	16.9
SPNO (m^{-2})	15742-38091	9642-26304	24859	18948	**	**	ns	20.0
PANO (m^{-2})	193-473	165-335	293	248	**	**	*	14.0
%FSP	75-92	76-97	86	89	**	**	**	5.5
GW (g)	17-27	16-26	21	20	**	**	**	6.0
FSPP	57-105	46-107	74	69	ns	**	ns	13.8
HI	0.35-0.55	0.39-0.59	0.49	0.53	**	**	*	6.0
%SN	0.38-0.72	0.46-0.72	0.57	0.59	ns	**	**	9.8
%GN	0.95-1.35	0.92-1.26	1.14	1.04	**	**	**	4.7
SN (g m^{-2})	1.6-3.4	1.2-2.1	2.5	1.6	**	**	ns	21.0
GN (g m^{-2})	3.1-6.2	1.7-4.4	5.0	3.5	**	**	*	20.9
TPN (g m^{-2})	4.8-9.1	3.1-6.0	7.5	5.2	**	**	ns	19.0
NUE	42-67	43-74	58	64	*	**	*	7.6
Medium								
GY (g m^{-2})	404-667	244-568	541	426	**	**	*	16.8
TDM (g m^{-2})	757-1124	502-890	972	704	**	**	*	15.0
SPNO (m^{-2})	19126-35425	12984-31525	25911	20339	**	**	**	19.0
PANO (m^{-2})	150-350	125-375	263	235	*	**	**	13.0
%FSP	72-95	75-95	86	89	**	**	**	4.8
GW (g)	16-25	15-26	21	20	*	**	**	6.0
FSPP	67-127	56-110	87	77	**	**	ns	14.7
HI	0.38-0.52	0.41-0.56	0.47	0.51	**	**	ns	6.0
%SN	0.41-0.63	0.36-0.71	0.52	0.51	ns	**	**	9.3
%GN	0.91-1.22	0.87-1.11	1.06	1.00	**	**	**	4.6
SN (g m^{-2})	1.5-4.1	1.2-2.3	2.5	1.7	**	**	**	19.0
GN (g m^{-2})	4.0-6.5	2.2-4.7	4.9	3.6	**	**	ns	18.2
TPN (g m^{-2})	5.7-9.5	3.8-6.9	7.5	5.4	**	**	**	16.0
NUE	47-71	54-82	62	67	**	**	**	7.7
Late								
GY (g m^{-2})	335-703	271-577	562	446	**	**	*	6.8
TDM (g m^{-2})	705-1322	623-1143	1078	791	**	**	*	14.3
SPNO (m^{-2})	15152-44416	13332-35653	27318	22035	**	**	ns	17.0
PANO (m^{-2})	150-375	125-325	269	242	*	**	*	13.2
%FSP	72-94	74-95	86	86	ns	**	**	4.4
GW (g)	13-28	11-27	21	20	**	**	**	4.1
FSPP	65-134	57-114	90	81	*	**	**	15.0
HI	0.34-0.51	0.37-0.55	0.45	0.45	**	**	**	5.7
%SN	0.38-0.67	0.38-0.64	0.50	0.47	**	**	**	9.0
%GN	0.91-1.46	0.91-1.40	1.06	1.03	*	**	**	4.9
SN (g m^{-2})	2.0-3.7	1.3-2.7	2.8	1.8	**	**	**	20.0
GN (g m^{-2})	3.3-6.2	2.9-4.8	5.1	4.0	**	**	ns	19.4
TPN (g m^{-2})	5.7-9.8	4.9-7.3	7.9	5.8	**	**	**	16.0
NUE	38-75	43-76	61	66	**	**	**	6.5

F, significance of F value from combined analysis of variance; Y, year; V, Variety; CV, coefficient of variation; ns, not significant at the 5% level.

groups, followed by HI, NUE, %SN, TILNO, and %GN with r greater than 0.5. On the other hand, data on TPN were not significantly correlated in years 1 and 2 (Table 4.4). Among the parameters measured, most genotypes showed significant changes

Table 4.4 Rank correlation between year 1 and year 2 data on selected plant parameters.

Parameter	Correlation coefficient		
	Early	Medium	Late
GW	0.863**	0.816**	0.944**
PLH	0.789**	0.792**	0.817**
PANO	0.719**	0.800**	0.766**
FSPP	0.772**	0.608**	0.629**
HI	0.595**	0.715**	0.662**
NUE	0.676**	0.628**	0.552**
%SN	0.661**	0.523**	0.661**
TILNO	0.570**	0.615**	0.575**
%GN	0.633**	0.605**	0.505**
SPNO	0.441**	0.610**	0.673**
%FSP	0.402**	0.542**	0.633**
GY	0.419**	0.371**	0.474**
TDM	0.354**	0.288**	0.449**
SN	0.407**	0.172^{ns}	0.290*
GN	0.369**	0.204^{ns}	0.201^{ns}
TPN	0.185^{ns}	0.083^{ns}	0.027^{ns}

^{ns} not significant at the 5% level.

between years in PLH, TDM, SN, and TPN (Table 4.5). Although PLH changed significantly in most genotypes between years, rankings were consistent. On the other hand, TPN decreased to very low levels in year 2 independent of their rankings in year 1. Less than 20% of the genotypes showed significant changes between years in FSPP, %SN and GW – traits with high rank correlation coefficients. The relative performance of genotypes in terms of NUE and HI were more consistent than TPN, TDM and GY in terms of rank correlations and number of genotypes which exhibited significant changes between years (Tables 3.7 and 3.8).

Scatter graphs of GY, TPN, and NUE data from 2 years show quantitatively the differential response of genotypes to change in available soil N between years (Fig. 4.1). There was a greater correlation in NUE than in N uptake between years. Thus, NUE appears to be a more stable and suitable criterion than N uptake.

Grain yield, TPN, HI, and NUE from 2-yr trials for each of the growth duration groups were subjected to principal component analysis. The first (PC1) and second (PC2) principal components accounted for 57 and 19% respectively of the total variability among genotypes in the early-duration group, 48 and 20% in the medium, and 50 and 17% in the late. In all the three growth-duration groups, PC1 was heavily loaded with GY, HI and NUE while PC2 was heavily loaded with N uptake. The

Table 4.5 Number of genotypes which showed significant ($P<0.05$) changes between year 1 and year 2 in selected plant parameters.

Parameter	Growth-duration group			Total
	Early	Medium	Late	
PLH	53	53	36	142
TDM	48	45	42	135
SN	44	48	45	137
TPN	48	45	38	131
GN	36	39	23	98
GY	25	31	30	86
%GN	41	23	20	84
SPNO	27	29	26	82
HI	25	23	24	72
NUE	21	19	24	64
TILNO	20	19	18	57
PANO	24	14	10	48
%FSP	10	11	15	36
GW	8	12	15	35
%SN	6	9	12	27
FSPP	3	12	11	26

variance (eigen value) of PC1 (early, 4.6; medium 3.8; and late, 4.0) in each group was significantly higher than that of PC2 (early, 1.5; medium, 1.6; and late, 1.4) while that of PC2 was not significantly different from PC3. Thus, PC1, which accounted for a major portion of the total variability in four parameters with a significantly higher variance than the succeeding principal components, were used to evaluate the relative performance of genotypes. Rankings based on PC1 were significantly correlated with GY rankings in both years with higher correlation coefficients (0.794-0.876) than that of GY ranks between years (Yable 3.7). Genotypes that consistently ranked high in GY and NUE in both years ranked high in PC1 (Table 4.6). IR13429-150-3-2-1-2 which ranked second among the early-duration genotypes also ranked high among 24 genotypes tested by De Datta and Broadbent (1990). Genotypes that ranked low in PC1 may be inefficient or inferior germplasm (Table 4.7). Further screening at higher N levels would clearly differentiate the inefficient from the inferior or agronomically unfit genotypes. Inferior genotypes would show only a slight increase in GY even with unlimiting supply of N.

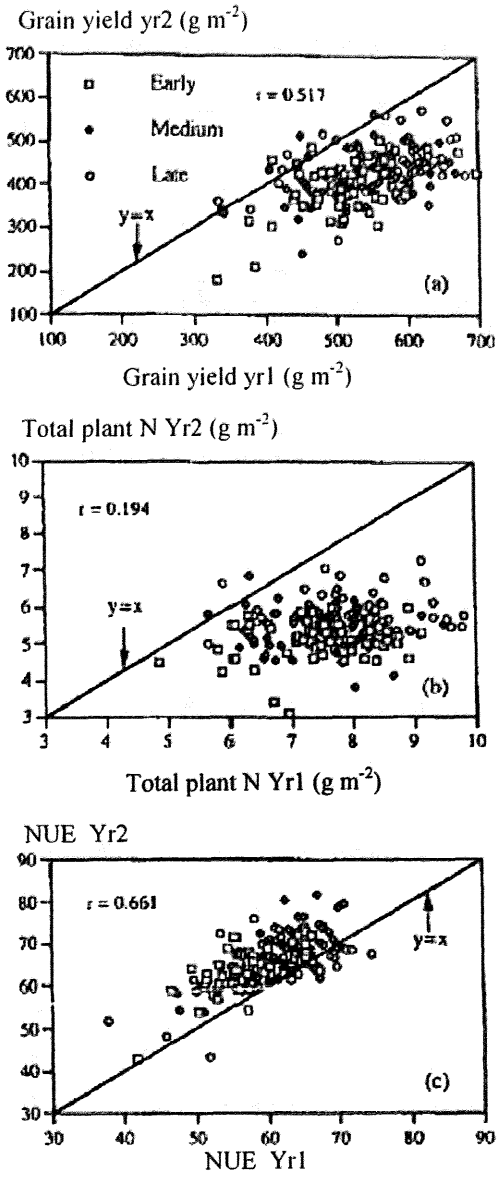


Fig. 4.1 Grain yield (a), N uptake (b) and NUE (c) in year 1 and year 2 in the early-, medium-, and late-duration genotypes.

Table 4.6 Genotypes in the early-, medium-, and late-duration groups having the highest first principal component (PC1) derived from GY, HI, NUE, and N uptake in year 1 and year 2.

Genotype no. [a]	Grain yield (Mg ha^{-1})		HI		NUE		N uptake (g m^{-2})		PC1
	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1
Early									
54	6.7	4.8	0.54	0.52	65.1	67.3	8.9	6.0	487
39	7.0	4.3	0.52	0.55	65.4	69.9	9.1	5.3	475
16	6.3	4,9	0.51	0.56	64.4	71.9	8.3	5.7	475
17	6.4	4.6	0.54	0.55	63.4	70.4	8.6	5.4	466
36	5.6	5.1	0.52	0.55	65.0	73.9	7.4	5.9	460
51	5.9	4.9	0.50	0.57	65.3	73.1	7.7	5.5	460
20	5.7	4.6	0.52	0.54	64.5	65.8	7.5	5.5	440
38	5.6	4.6	0.53	0.58	64.0	72.2	7.3	5.4	439
31	5.8	4.9	0.49	0.52	59.5	63.1	8.3	6.0	437
44	5.5	4.7	0.48	0.54	55.5	71.6	8.2	5.5	435
Medium									
89	6.5	5.0	0.52	0.54	67.2	70.4	8.3	6.1	488
95	6.3	4.9	0.48	0.54	67.4	74.0	8.0	5.7	478
78	6.6	4.6	0.50	0.55	70.0	78.8	8.0	5.0	475
67	6.0	5.1	0.49	0.52	66.3	67.0	7.8	6.5	474
77	6.1	5.0	0.52	0.55	66.9	81.9	8.2	5.2	474
75	6.1	4.9	0.47	0.52	65.8	67.5	8.0	6.2	470
99	6.7	4.3	0.52	0.55	68.7	72.8	8.4	5.1	465
111	5.5	5.2	0.47	0.53	58.9	72.5	8.1	6.0	459
68	6.2	4.6	0.49	0.50	63.8	65.8	8.4	6.0	459
103	5.8	4.7	0.51	0.54	69.2	71.0	7.2	5.7	449
Late									
163	6.6	5.5	0.43	0.46	69.7	69.8	8.6	6.8	555
126	6.2	5.8	0.50	0.53	70.7	69.2	7.6	7.1	549
162	7.0	4.9	0.49	0.51	64.8	68.9	9.3	6.2	547
129	6.7	5.1	0.46	0.55	58.2	75.9	9.8	5.8	542
173	6.6	5.1	0.47	0.51	61.8	70.1	9.2	6.7	537
172	5.9	5.5	0.48	0.50	65.1	68.1	7.8	6.9	525
158	6.5	4.7	0.50	0.53	67.3	74.8	8.2	5.4	517
125	6.0	5.2	0.47	0.49	68.8	67.2	7.2	6.5	514
168	5.7	5.6	0.38	0.42	53.7	64.7	9.1	7.3	514
151	6.8	4.3	0.47	0.50	63.9	66.2	9.8	5.5	511

[a] See Table 3.1 for genotype identity

Table 4.7 Genotypes in the early-, medium-, and late-duration groups having the lowest first principal component (PC1) derived from GY, HI, NUE, and N uptake in year 1 and year 2.

Genotype no.[a]	Grain yield (Mg ha^{-1})		HI		NUE		N uptake (g m^{-2})		PC1
	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1
Early									
59	3.3	1.8	0.38	0.39	41.8	43.1	6.7	3.4	223
24	3.8	2.1	0.35	0.43	46.5	58.9	7.0	3.1	262
33	3.8	3.2	0.46	0.51	53.0	57.1	6.1	4.6	305
55	3.4	3.4	0.39	0.46	60.2	64.4	4.8	4.5	308
46	4.1	3.0	0.49	0.53	59.0	63.7	5.9	4.2	315
6	4.9	3.2	0.54	0.48	57.1	54.3	7.4	4.9	346
3	4.5	3.5	0.44	0.48	51.1	59.7	7.6	5.0	347
26	5.1	3.1	0.49	0.50	50.3	54.0	8.5	5.0	348
40	4.4	3.6	0.46	0.51	53.4	59.6	7.1	5.1	349
27	4.7	3.6	0.49	0.50	54.3	60.7	7.5	4.9	358
Medium									
66	4.5	2.4	0.38	0.41	47.8	54.5	8.0	3.8	294
64	4.4	3.2	0.47	0.51	56.3	58.9	6.7	4.5	329
101	4.3	3.5	0.42	0.43	51.3	53.8	7.0	5.6	334
72	4.6	3.5	0.47	0.48	60.2	60.5	6.6	4.9	351
65	4.3	3.9	0.48	0.53	59.5	67.2	6.1	4.9	358
98	4.6	4.0	0.40	0.48	47.4	58.3	8.5	5.8	364
61	5.1	3.4	0.51	0.49	67.2	61.8	6.5	4.6	366
100	4.0	4.4	0.42	0.50	55.8	61.8	6.3	6.1	368
109	5.1	3.5	0.46	0.48	57.4	59.1	7.7	5.1	368
116	4.5	4.1	0.46	0.52	61.9	65.5	6.2	5.4	375
Late									
148	3.4	3.3	0.43	0.45	45.8	48.2	6.5	5.9	317
134	3.4	3.6	0.34	0.42	49.8	61.4	5.7	5.0	331
145	3.8	3.4	0.36	0.40	37.9	52.0	7.6	5.6	333
146	5.0	2.7	0.43	0.37	52.1	43.3	8.7	5.4	359
149	4.2	4.1	0.45	0.51	54.3	61.0	6.6	5.6	386
169	4.7	3.8	0.35	0.38	53.1	59.2	7.5	5.6	397
135	4.6	4.0	0.45	0.49	58.6	60.8	6.4	5.6	402
178	4.2	4.4	0.37	0.46	55.3	64.5	6.5	5.7	402
175	5.3	3.6	0.43	0.49	59.7	63.0	7.7	4.9	415
122	4.9	4.0	0.46	0.50	57.9	60.5	7.3	5.7	416

[a] See Table 4.1 for genotype identity

4.5.2 Genotype variability in grain yield as affected by N uptake and NUE

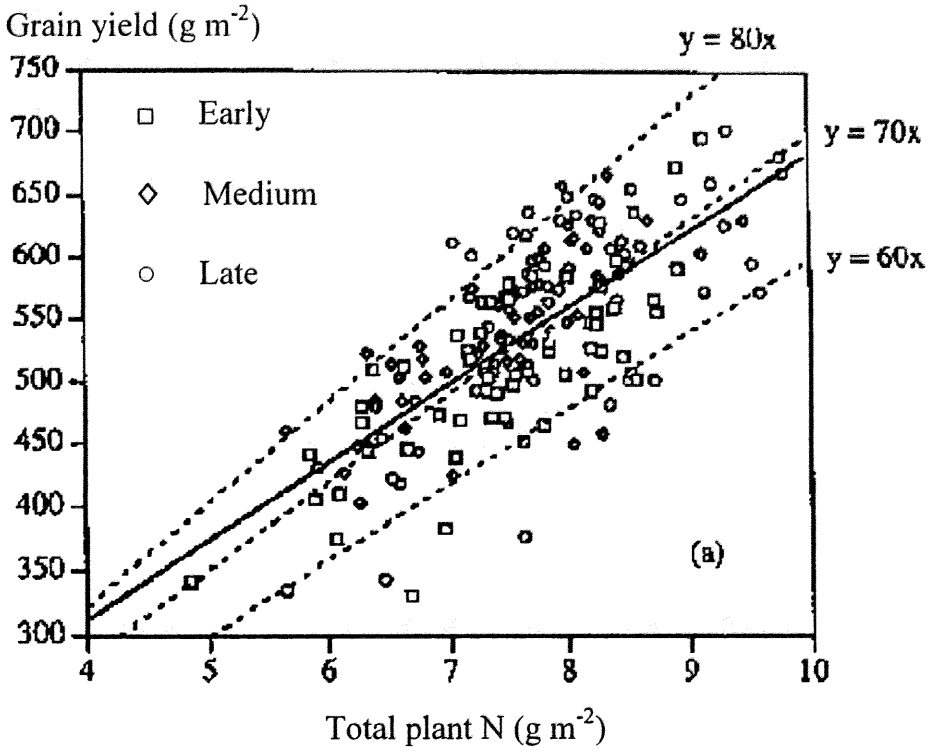
Grain yield was highly correlated with total plant N. Based on the slope of the regression curve, the average increase in grain yield for each kilogram increase in soil N uptake was 61.9 kg in year 1 and 82.7 kg in year 2 for the 180 genotypes tested, demonstrating a higher yield gain per unit increase in N uptake under lower soil N

(Fig. 4.2). The average NUE computed was 60 for year 1 and 66 for year 2 (Table 4.3). Singh et al. (1995) observed a yield increase of 55.5 kg for each additional kg increase in soil N uptake during the dry season from irrigated plots without N fertilization in another field at the IRRI farm. The value obtained in this study for year 2 was much higher due to a very low soil available N. Although a linear relationship may be observed between GY and TPN at suboptimal N levels, this relationship becomes quadratic over a wider range of N levels (Cassman et al., 1993). This can explain why the slope of the regression curve and the correlation coefficient between GY and TPN are both higher in year 2 than in year 1 (Fig. 4.2).

4.6 Discussion

Results show a higher correlation of TPN with growth duration in the late-duration genotypes during the second year when soil available N was lower. During the second year, N uptake in 25% of the genotypes in the late duration group were greater than the maximum observed in the early group, whereas during the first year, when soil available N was higher, only five out of the 60 genotypes in the late-duration group had taken up more N than the one which absorbed the most N in the early group. These observations suggest a greater dependence of plant N uptake on growth duration when N is limiting. Moreover, during the second year, most of the genotypes in the early group absorbed similar amounts of N ranging from 4.5 – 6.0 g m^{-2}, excluding the traditional and upland varieties. Greater differences were observed among modern varieties in the late duration group with a range of 4.9 – 7.3 g m^{-2}. A better adaptation to suboptimal N condition may be found among long-duration cultivars which can extract more soil N during their extended growing period. During the second year, NUE was not significantly correlated with growth duration (Table 4.2). Thus, the higher GY obtained by long duration genotypes may be attributed principally to their higher N uptake.

Genotypes differed in their response to the change in soil available N as shown by the significant ($P<0.05$) genotype x year interaction from ANOV for most of the parameters measured (Table 4.3). The small variation in solar radiation and temperature between years was not enough to have caused any significant change in the relative performance of the genotypes. The average daily solar radiation coinciding with the vegetative phase was 17±5 in year 1 and 18±5 MJ m^{-2} in year 2, while that coinciding with the grain-filling period was 21±3 in year 1 and 22±3 MJ m^{-2} in year 2. When yields were simulated using the CERES-Rice model (Singh et al., 1993), a decline in yield was predicted from year 1



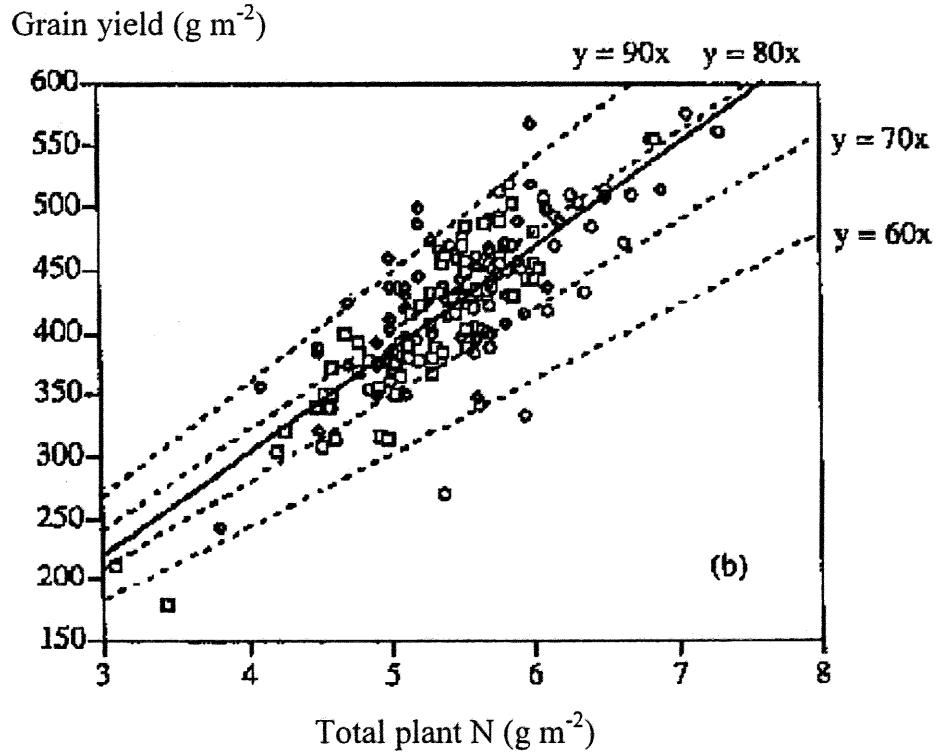


Fig. 4.2 Grain yield vs. total N uptake in the early-, medium-, and late-duration genotypes in year 1(a) and year 2(b). In year 1, $y = 61.9x + 66$, $r = 0.731^{**}$. In year 2, $y = 82.7x - 28$, $r = 0.812^{**}$. The broken lines may be described by the equation $y = mx$, where m = slope = NUE/0.86.

to year 2 under zero N, principally due to lower soil N in year 2. The model predicted yields of 5.9 and 3.1t ha^{-1} for years 1 and 2 respectively under zero N, and 7.8 and 8.0 t

ha^{-1} for the first and second years when N is nonlimiting, for variety IR50 with a population of 25 plants m^{-2}. This shows a yield increase relating to climatic factor (solar radiation) under nonlimiting conditions of only 2.6% or 0.2 t ha^{-1} against a decline in yield of 47% or 2.8 t ha^{-1} under zero N. The actual yields obtained for IR50 were 5.2 t ha^{-1} in year 1 and 3.9 t ha^{-1} in year 2. These results support the conclusion that the major difference between the 2 years was soil N availability.

Among the parameters measured TPN appears to be the least stable based on the responses of genotypes in years 1 and 2. The differential response of genotypes to the change in available soil N in terms of N uptake or N acquisition efficiency [δN uptake $_{(yr1-yr2)}$ / δavailable $N_{(yr1-yr2)}$] may be influenced by the amount of available soil N; varietal traits e.g. increased root length density and higher absorption per unit length that enable plants to take up N from different soil layers (Kundu and Ladha, 1995), stimulate biological N_2-fixation (Ladha et al, 1988) or influence soil N mineralization; synchrony of crop demand and soil N supply; and crop, water and nutrient practices (Cassman et al., 1994). Crop, water and nutrient management practices were essentially the same in year 1 and year 2, so these could not have influenced differences in N acquisition in these trials.

Breeders usually gauge the performance of genotypes by their GY. De Datta and Broadbent (1988) obtained the sum of Z-transformed values of GY, NUE, panicle weight (PW), PW/TPN, and total dry matter (TDM)/TPN, putting equal weights on each parameter, to rank genotypes. However, these parameters may not be of equal importance. Among the five parameters, they selected GY and PW/TPN, which gave the greatest variability among genotypes, and determined their coefficients by multiple regression analysis using the 5-parameter sum as the dependent variable. They obtained R^2 (coefficient of determination) of 0.9 or greater and rankings that were similar to the 5-parameter rankings. Fewer genotypes belonging to a homogenous group of varieties and breeding materials were used and significant difference in N uptake was observed in only one season.

In this study, GY, TPN, HI, and NUE were used to assess the overall performance of genotypes as these are the end results of the interaction of several parameters: yield components – spikelet weight and ripening percentage, straw dry weight, root dry weight, %GN, %SN, GN, SN, TILNO etc. Grain yield and TPN reflect the absolute gains while HI and NUE are efficiency terms. Much of the variation in NUE appears to be associated with differences in HI. Generally, traditional varieties have lower HI and NUE than modern high-yielding varieties, with equivalent amounts of N taken up and total biomass

produced. However, differences in NUE may also be observed among genotypes with similar HI. A genotype having high GY, TPN, HI and NUE would be most desirable. Although these four variables were correlated with each other, rankings of genotypes based on each of them were not the same.

4.6.1.2 Genotype variability in grain yield as affected by N uptake and NUE

As a general trend, most cultivars increased in GY with increase in TPN. Some points falling on the same $y = mx$ line (where m = slope = NUE/0.86, Fig. 4.2), were identified showing significant differences in GY and N uptake but not NUE (Table 4.8). These

Table 4.8 Selected genotypes in the early-, medium-, and late-duration groups that showed significant differences in grain yield and N uptake but not NUE.

Genotype no.[a]	Grain yield (Mg ha^{-1})		HI		NUE		N uptake (g m^{-2})	
	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
Early								
39	7.0*	4.3	9.1*	5.3	65.4	69.9	0.52	0.55
9	4.4	3.8	5.8	4.8	65.4	66.9	0.52	0.58
7	5.6*	4.4	9.1*	6.0	52.3	62.9	0.48	0.54
33	3.8	3.2	6.1	4.6	53.0	57.1	0.46	0.51
54	6.7*	4.8	8.9*	6.0	65.1	67.3	0.54	0.52
30	4.8	4.3	6.3	5.4	65.3	68.6	0.52	0.58
Medium								
99	6.7*	4.3	8.4*	5.1	68.7	72.8	0.52	0.55
61	5.1	3.4	6.5	4.6	67.2	61.8	0.51	0.49
89	6.5*	5.0	8.3*	6.1	67.2	70.4	0.52	0.54
73	4.6	4.7	5.7	5.8	69.6	69.1	0.51	0.54
75	6.1*	4.9	8.0*	6.2	65.8	67.5	0.47	0.52
85	4.8	3.9	6.4	5.1	64.4	65.1	0.46	0.48
Late								
173	6.6*	5.1	9.4*	6.7	61.8	70.1*	0.47	0.51
135	4.6	4.0	6.4	5.6	58.6	60.8	0.45	0.49
152	6.3*	4.5	9.3*	5.8	57.9	64.0	0.45	0.47
149	4.2	4.1	6.6	5.6	54.3	61.0	0.45	0.51
129	6.7*	5.1	9.8*	5.8	58.2	75.9*	0.46	0.55
136	5.1	4.0	7.3	5.2	59.8	65.4	0.43	0.46

[a] See Table 3.1 for genotype identity.
* Value is significantly different from the one below it at the 5% level.

genotypes may differ in their ability to take up soil N because of diverse plant characteristics as discussed earlier. However, some genotypes absorbed similar amounts of N but produced different GYs and / or total dry matter as illustrated by the points scattered above and below the regression line (Fig. 4.2). This is an indication that

genotypes vary in their ability to utilize N. Significant differences in %SN at maturity were observed in these genotypes (Table 4.9). For genotypes with similar HI, a lower straw N concentration may indicate higher NUE. Straw N concentration was not significantly affected by the change in available soil N in the early- and medium-duration groups. Moreover, there was a high rank correlation in %SN measurements between years in all growth-duration groups (Table 4.4).

Table 4.9 Selected genotypes in the early-, medium-, and late-duration group that showed similar N uptake but different grain yield and /or total biomass.

Genotype no.[a]	Grain yield (Mg ha^{-1})		Total biomass (mg ha^{-1})		N Uptake (g m^{-2})		% Straw N		NUE		HI	
	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2	Yr1	Yr2
Early												
16	6.3*	4.9	10.6*	7.4	8.3	5.7	0.57*	0.55*	64.4*	71.9*	0.51	0.56
5	4.7	3.8	8.3	6.2	7.4	5.3	0.68	0.69	55.3	60.8	0.49	0.53
54	6.7*	4.8	10.7*	7.9	8.9	6.0	0.52*	0.51*	65.1*	67.3*	0.54*	0.52
40	4.4	3.6	8.1	6.0	7.1	5.1	0.68	0.69	53.4	59.6	0.46	0.51
39	7.0*	4.3	11.5*	6.9	9.1	5.3	0.55*	0.52*	65.4*	69.9*	0.52	0.55
37	4.9	4.3	8.8	6.5	8.2	5.9	0.72	0.64	51.8	61.5	0.48	0.57
Medium												
78	6.5*	4.6	11.1*	7.2*	8.0	5.0	0.52*	0.47*	70.0*	78.8*	0.50	0.55
64	4.4	3.2	8.0	5.2	6.7	4.5	0.63	0.71	56.3	58.9	0.47	0.51
75	6.1*	4.9	11.2*	8.0	8.0	6.2	0.43*	0.44*	65.8	67.5	0.47	0.52
72	4.6	3.5	8.5	6.2	6.6	4.9	0.58	0.62	60.2	60.5	0.47	0.48
88	5.3	5.0	11.1*	8.9	7.3	6.1	0.42*	0.41	62.2	70.0	0.41*	0.48
80	4.8	4.4	8.5	7.0	6.4	5.0	0.53	0.47	64.9	73.9	0.49	0.52
Late												
163	6.6*	5.5*	13.2*	10.4*	8.5	6.8	0.43*	0.43*	69.7*	69.8*	0.43	0.46
122	4.9	4.0	9.1	6.9	7.3	5.7	0.54	0.56	57.9	60.5	0.46	0.50
177	6.0*	3.9	11.9*	7.6	7.8	5.1	0.43*	0.40	65.8*	65.9*	0.43	0.44
148	3.4	3.3	7.0	6.3	6.5	5.9	0.53	0.54	45.8	48.2	0.43	0.45
155	5.7*	4.7	10.9*	8.2	7.2	5.9	0.43*	0.44*	71.8*	68.6	0.45	0.49
149	4.2	4.1	8.2	6.8	6.6	5.6	0.53	0.53	54.3	61.0	0.45	0.51

[a] See Table 3.1 for genotype identity.
* Value is significantly different from the one below it at the 5% level.

Differences in N use may occur because of differences in critical concentration (internal N requirement) for expansion growth, mass accumulation and organ formation (Singh and Buresh, 1994) and/or due to differences in the ability to translocate (rate of radial and axial flow of acquired N), distribute N to various sinks, remobilize from older organs to newer ones, and partition absorbed N to various organs and products (straw N vs. grain N) at maturity (Ladha et al., 1993).

Differences in GY among genotypes at suboptimal N levels may be attributed to variability in both N uptake and NUE. Efficient genotypes may be described as those which produce high grain yields at suboptimal N levels through increased N uptake and/or a more efficient utilization of the N taken up for grain production. A lower straw N concentration at maturity may indicate higher NUE. Straw N concentration of a genotype at maturity may not be significantly affected by the change in available soil N. Grain N concentration also decreased by an average of 0.1% as NUE increase by 10. Hence the nutritional value of rice is not really sacrificed with the improvement of NUE. Grain N concentration was not greatly affected by the change in soil N supply.

It appears that NUE is a more stable and suitable selection criterion than N uptake. However, there is a need to identify a simple, easily measured parameter, based on physical, phenotypic traits which could be a good indicator of NUE. Efficient genotypes are expected to show highest potential benefit under suboptimal (low-input) agriculture. Moreover, screening of genotypes for NUE at low N levels would avoid problems due to pests and diseases and lodging which create artifacts at high N levels. Genotypes with superior NUE and consistently good yield at suboptimal N levels have been identified. Further assessment of their yield performance and N uptake patterns at higher N levels and different sites is needed to test their stability and elucidate the mechanism for N uptake difference.

CHAPTER 5

INTEGRATING PRODUCTIVITY TRENDS IN RICE-RICE AND RICE-WHEAT SYSTEMS AS AFFECTED BY INORGANIC AND ORGANIC FERTILIZER MANAGEMENT

5.1 Summary

Long-term trends of crop yields have been used as a means to evaluate the sustainability of intensive agriculture. Previous studies have measured yield trends from long-term rice-rice and rice-wheat experiments in different sites from the slopes of individual site regressions of yield over time. The statistical significance of each site regression was determined but not that of the aggregate trend, which could give an indication of the magnitude and significance of global yield change.

The random regression coefficient analysis (RRCA) and meta-analysis were used in this study to analyze the aggregate yield trend from several long-term experiments (LTE) across the Indo-Gangetic Plains (IGP) and outside the IGP. Both methods show that there has been a significant ($P<0.05$) declining trend in rice yield in rice-wheat LTEs in South Asia including China with the recommended rates of nutrients, but that there has been no significant change in wheat and system (rice+wheat) yields. There was no significant year x region (IGP vs. non-IGP) interaction in rice and wheat yields. However, RRCA showed that the average yield trend was significantly negative (−41.0 kg ha^{-1} yr^{-1}) only in the IGP. In the rice-rice LTEs, there was a significant year x site (IRRI vs. non-IRRI sites) interaction during the dry season but not the wet season. Rice yields declined throughout Asia in the wet season. The average system (dry+wet season rice) yield trends were significantly negative in both IRRI and non-IRRI sites (−170.1 and −52.8 kg ha^{-1} yr^{-1}, respectively) but the magnitude of yield decline was significantly greater in the IRRI sites than in the non-IRRI sites.

Rice in the rice-wheat LTEs showed a significantly positive yield trend with the addition of farmyard manure (FYM) but the initial yield was generally lower with FYM than without FYM. After 15 years, yield increase due to FYM was not evident in most of the LTE.

5.2 Introduction

Monitoring long-term changes in crop yields is essential in evaluating sustainability of intensive agriculture. Long-term experiments (LTE) provide the only direct method to

determine the sustainability of new management systems, and the efficacy of measures used to enhance productivity. In the 1960s, a new generation of short-duration rice and wheat varieties were introduced throughout Asia. These varieties, which promoted crop intensification together with widespread use of fertilizers and other chemical inputs led to the Green Revolution. Many long-term fertilizer and manure experiments measuring the sustainability of input-intensive double- and triple-cropped monoculture rice and dual culture rice-wheat systems started almost at the same time.

Extensive efforts have been made to analyze the long-term trends of crop yields, although in isolated cases with different statistical approaches, conflicting conclusions in relation to yield trends have been drawn (Nambiar, 1994; Nambiar and Abrol, 1989; Swarup et al., 1989; Abrol et al., 2000). Recently, systematic efforts have been made to make global and regional interpretations using combined analysis with the most recent data and identical statistical procedures. Dawe et al. (2000) and Ladha et al. (2003) analyzed yield trends from over 50 long-term experiments within rice-rice or rice-wheat systems from 7 countries. Using the slopes from individual site regressions of yield over time, conclusions were made based on the number of LTE with positive and negative trends, or the number with significant *p*-values. However, *p*-values cannot be used to measure the magnitude of treatment effects and are dependent on the number of data points. Unless the effect is huge, it is difficult to obtain a significant *p* value from an experiment with only a few data points. Experiments with a large number of data points are more likely to exhibit significant *p* values than those with few data points, although they may show similar trends. Thus, the statistical significance of the overall trend cannot simply be determined by comparing the number of significant and non-significant *p* values. The RRCA computes the average trend and its statistical significance by combining data from different experiments taking into account the number of data points and variance in each experiment. By combining all data, the sample size increases and the confidence in the overall result also increases.

The RRCA can be performed using the SAS linear mixed-effects model (LMEM), which has been used for analyzing longitudinal data (Goldstein, 1995; Pollack, 1998). Longitudinal data consist of measurements made on the same subjects repeatedly over time (Goldstein, 1979). The LMEM is well suited for data from a multi-strata structure with different levels of variability. In the LMEM, both fixed (affects the population mean) and random (associated with the individual) effects occur linearly in the model

function (Pinheiro and Bates, 2000). Linear mixed-effects models make prediction curves for population (e.g., overall sites) and individuals (e.g., for each site).

Meta-analysis is another statistical tool that integrates findings from a collection of studies and describes the results using numerical effect-size estimates (SAS Institute Inc. et al., 1999). There are two major classes of effect sizes (a) variance-accounted-for effect sizes analogous to a squared correlation coefficient, and (b) standardized mean differences (Rosenthal, 1994). Normally, a population effect size is estimated by averaging standardized or weighted effect size estimates from the individual studies. Effect size estimates are standardized using the inverse of their variances giving studies with larger sample sizes more weight in the average estimate. The SAS macro WAVGMETA produces an estimate and 95% confidence intervals based on this weighted average procedure (SAS Institute Inc., 1999).

5.3 Objectives

The objectives of this study were to examine:

a. the global magnitude of rice and wheat yield change and its significance by doing a combined analysis of data from more than 50 rice-rice and rice-wheat LTE across Asia; and
b. regional differences in fertilizer management effects on yield trends

5.4 Materials and Methods

The data analyzed in this study were taken from 33 rice-wheat long-term experiments conducted in 24 different sites in India (17 sites), Nepal (3 sites), Bangladesh (1 site), and China (3 sites), which ran from 7 to 24 yrs; and 23 rice-rice long-term experiments in 17 different sites in the Philippines (6 sites), China (3 sites), Indonesia (3 sites), India (1 site), Bangladesh (2 sites), Malaysia (1 site), and Vietnam (1 site), which ran from 10 to 24 yrs. Descriptions of the rice-wheat experimental sites are in Table 5.1 and the rice-rice experimental sites are in Dawe et al.(2000). In all the LTE, data from treatments with the recommended doses of NPK were analyzed. In 19 LTE, treatments with FYM in combination with NPK fertilizer were also analyzed to determine the effect of FYM on yield trends. The inorganic NPK combined with FYM was at most equal or at least half of the NPK added in the 100% inorganic NPK treatment.

The annual yield data from LTE in different sites were combined and the average trend and its significance were analyzed using random regression coefficient analysis (RRCA).

This was performed using the SAS procedure MIXED for linear mixed models or the LMEM. We also considered fitting other covariance structures for the yields measured in the same location over time. However, the random regression coefficient approach followed the best fit.

The independent variables in the model were year, region, and year x region interaction. By including the year x region interaction in the model, we were able to estimate and compare the yield trends in rice-wheat systems in the IGP and non-IGP and yield trends in rice-rice systems in IRRI and non-IRRI sites.

A similar analysis was performed to compare rice yield trends in rice-wheat systems with and without farmyard manure in combination with NPK. The model included year, treatment, and year x treatment interaction.

Meta-analysis was done using the SAS macro WAVGMETA (SAS Institute Inc., 1999), which computes the weighted average of sample correlations. The correlation coefficient (r) or the association between grain yield and the number of cropping years in each experiment (i.e. grain yield during the n^{th} year vs. n) was used as effect size estimate. A dot plot (Cleveland, 1985) was used to display effect-size estimates (with their 95% confidence limits) separately for each study. Unless indicated otherwise, differences were considered significant only when $P \leq 0.05$. The value of the correlation indicates the strength of the relation. Cohen (1988) suggested that a correlation of 0.5 is large, 0.3 is moderate, and 0.1 is small.

A system yield refers to the total yield harvested annually from 2 cereals together in a cropping system (rice-rice or rice-wheat).

5.5. Results

5.5.1 Productivity trends in rice-wheat systems

5.5.1.1 Integrating rice and wheat productivity trends using randomized regression coefficient analysis

The average yield trend in 33 LTE across South Asia and China was found to be significantly negative [-28.6, 95% confidence limit (CL) = −56.9 to −0.3 kg ha^{-1} yr^{-1}]. On the other hand, wheat yield was stable, i.e., the aggregate trend or slope was not significantly different from 0 (Table 1).

Ladha et al. (2003) partitioned the LTE conducted in Asia (South Asia and China) into (a) Indo-Gangetic Plains (IGP), (b) outside IGP with China (non-IGP),

Table 5.1 Long-term rice-wheat and rice-rice-wheat experiments in South Asia and China

LTE No.	Location			Experiment ID[a]	Soil type[b] (Texture class)	Fertilizer rate[c] (N-P-K rice; N-P-K wheat)	Organic Fertilizer[d]	Duration[e]	Reference[f]
	IGP	Country	Site						
1	2	India	Ludhiana 1	AICRP-CSR	Typic Ustochrept (LS)[h]	120-26-25; 120-26-25	None	1984-2001	Bhandari et al., 2002; Yadav et al., 2000b
2	2	India	Ludhiana 2	Residue	Ustochrept (LS)	120-30-30; 120-60-30	Residue	1993-2000	Not available
3	2	India	Ludhiana 3	Phosphorus	Typic Ustochrept (LS)	150-26-25; 120-26-25	None	1991-2001	Bijay Singh et al., 2000; Dawe et al., 2000
4	2	India	Ludhiana 4	Residue	Typic Ustipsamment (LS)	150-0-0; 150-26-50	None	1988-2000	Yadvinder Singh et al., 2000
5	2	India	Karnal	Phosphorus	Aquic Natrustalf	120-22-50; 120-22-50	None	1976-1996	Chhabra and Thakur, 2000[g]
6	2	India	Karnal1	AICRP-CSR	Aquic Natrustalf	120-22-42; 120-22-42	None	1974-1986	Singh and Swarup, 2000; Dawe et al., 2000
7	3	India	Pantnagar 1	AICRP-CSR	Aquic Hapludoll (SiCL)	120-26-37; 120-26-37	FYM	1972-1992	Ram, 2000
8	3	India	Pantnagar 2	LTE2(b)	Typic Hapludoll (L)	120-35-33; 120-35-33	None	1977-2000	Yadvider Singh et al., 2000; Dawe et al., 2000
9	3	India	Pantnagar 3	AICRP-CSR	Hapludoll (SiL)	120-26-33; 120-26-33	None	1983-2000	Yadav et al., 2000b
10	3	India	Pantnagar 4	LTFE	Fluventic Haplaquoll (SiL)	120-17-33; 120-17-33	None	1984-1999	Dawe et al., 2000
11	3	India	Kanpur	AICRP-CSR	Udic Ustochrepts	120-26-33; 120-26-33	None	1984-2000	Yadav et al., 2000b
12	3	Nepal	Nepalgunj	LTSFE	Dystric eutrochrept (SiL)	100-30-30; 100-30-30	None	1978-1998	Bhattarai and Mishra, 1999
13	4	India	Faizabad	AICRP-CSR	Udic Fluaquents (SiL)	120-26-33; 120-26-33	None	1984-1999	Yadav et al., 2000b
14	4	India	Varanasi	AICRP-CSR	Aeric Ochroqualfs	120-26-33; 120-26-33	None	1985-2000	Yadav et al., 2000b
15	4	India	Pusa		Ustochrept	150-33-60; 150-33-62	None	1985-1999	Sakal, 2000[g]
16	4	India	Sabour	AICRP-CSR	Ustochrept (Clayey)	120-26-33; 120-26-33	None	1984-2000	Not available
17	4	Nepal	Bhairawa 1	LTSFE	Typic Haplaquepts (SL)	100-13-25; 100-18-25	None	1979-2000	Regmi et al., 2002a
18	4	Nepal	Bhairawa 2	LTSFE	Typic Haplaquepts (SL	100-13-0; 100-13-0	FYM	1988-2000	Regmi et al., 2002b
19	4	Nepal	Parwanipur	LTSFE	Inceptisol (LS)	100-13-25; 100-13-25	None	1980-2000	Gami et al., 2001
20	4	Nepal	Tarahara	LTSFE	Typic Haplaquepts (L)	100-13-25; 100-17-25	None	1978-1997	Yadav et al., 1999
21	5	India	Barrackpore	AICRP-LTE	Eutrochrept (SL)	180-39-75; 180-39-75	None	1973-1997	Saha, 2000; Dawe et al., 2000

22	5	India	Kalyani	AICRP-CSR	Udic Ustochrepts	120-26-33; 120-26-33	None	1986-2000	Yadav et al., 2000b
23	5	Bangladesh	Dinajpur		na[i]	135-80-120; 180-80-120	None	1992-2001	Badaruddin et al., 2000
24	Non-IGP	India	RS Pura	AICRP-CSR	Eutrochrept (CL)	120-26-33; 120-26-33	None	1985-2000	Not available
25	Non-IGP	India	Palampur 1	AICRP-CSR	Udic Haplustalf	120-26-33; 120-26-33	None	1985-2000	Not available
26	Non-IGP	India	Palampur 2	LANTANA	Typic Hapludalf (SiCL)	90-18-33; 120-26-25	None	1988-2000	Sharma et al., 2002
27	Non-IGP	India	Rewa		(SiCL)	120-35-33; 120-35-33	None	1977-2000	Singh and Khan, 2000[g]
28	Non-IGP	India	Jabalpur	AICRP-CSR	Chromoustert (Clayey)	120-26-33; 120-26-33	None	1985-2000	Not available
29	Non-IGP	India	Raipur	AICRP-CSR	Ochraqualfs (Clayey)	120-26-33; 120-26-33	None	1988-1999	Not available
30	Non-IGP	India	Navsari	AICRP-CSR	Vertic Ustochrept	120-26-33; 120-26-33	None	1987-2000	Not available
31	China	China	Nangong	LTFE	Paddy soil, river alluvium (SiCL)	120-17-33; 120-17-33	None	1983-1996	Dawe et al., 2000
32	China	China	Sichuan	LTE	Purple paddy soil (L)	113-25-47; 188-41-78	None	1984-1998	Dawe et al., 2000
33	China	China	Jiangsu	LTE	Sandy loam	150-19-90; 150-23-90	None	1985-1996	Zhuang et al., 1999

[a]AICRP-CSR, All India Coordinated Research Project on Cropping Systems Research; LTE, Long-Term Experiment; LTFE, Long-Term Fertilizer Experiment; LTSFE, Long-Term Soil Fertility Experiment.

[b]Soil classification using USDA Soil Taxonomy (Soil Survey Staff, 1994).

[c]Fertilizer rates given on elemental basis are the recommended rates which produced the yields reported in tables 2-5. The LTEs involve other treatments representing different levels of mineral and organic fertilizer. For each experiment, the first row shows fertilizer rates for rice, the second row for wheat.

[d]Organic fertilizer applied together with inorganic NPK resulting in the yields reported in tables 2-5. Residue refers to rice or wheat straw; FYM in Pantnagar 1 was added at 15 Mg ha^{-1}; FYM in Bhairawa 2 was added at 4 Mg ha^{-1}.

[e]Duration of the experiment.

[f]paper describing more experimental details and results.

[g]Data of the LTE was obtained from this publication.

[h]LS, loamy sand; L, loam; SL, sandy loam; CL, clay loam; SiL, silty loam; SiCL, silty clay loam

[i]Not available

and (c) all together (Asia). The average yield trends from separate regression analyses were determined but tests of significance were done only on individual site trends. In this study, the same groupings were followed to determine the year x region interaction on the aggregate rice and wheat yields (yield trends) and their levels of significance. There was no significant year x region interaction in rice and wheat yields. However, the average rice yield trend in the IGP was significantly negative (−40.9, 95% CL = −74.5 to −7.4 kg ha^{-1} yr^{-1}) while there was no significant trend in the non-IGP (Table 1).

In wheat, the average yield change was not significantly different from zero in either the IGP or non-IGP. Since wheat yields were stable, the change in rice-wheat system yields across sites was also not significant (Table 5.2).

Table 5.2 Random regression coefficient analysis of yield trends in 33 rice-wheat LTE performed using the SAS procedure MIXED for linear mixed models.

Sites	No. of LTEs	Slope kg ha^{-1} yr^{-1}	Pr > |t|	Lower 95% CL[a] kg ha^{-1} yr^{-1}	Upper 95% CL[a] kg ha^{-1} yr^{-1}
Rice					
All sites	33	−28.6	0.05	−56.8	−0.3
Yr * region			0.28[b]		
IGP	23	−41.0	0.02	−74.5	−7.4
Non-IGP	10	−6.1	0.82	−61.1	48.9
Wheat					
All sites	33	2.7	0.81	−20.9	26.2
Yr * region			0.68[b]		
IGP	23	3.7	0.77	−22.2	29.8
Non-IGP	10	−7.4	0.75	−54.4	39.7
System					
All sites	33	−26.9	0.14	−63.3	9.6
Yr * region			0.46[b]		
IGP	23	−34.0	0.11	−76.2	8.1
Non-IGP	10	−23.7	0.95	−77.0	72.3

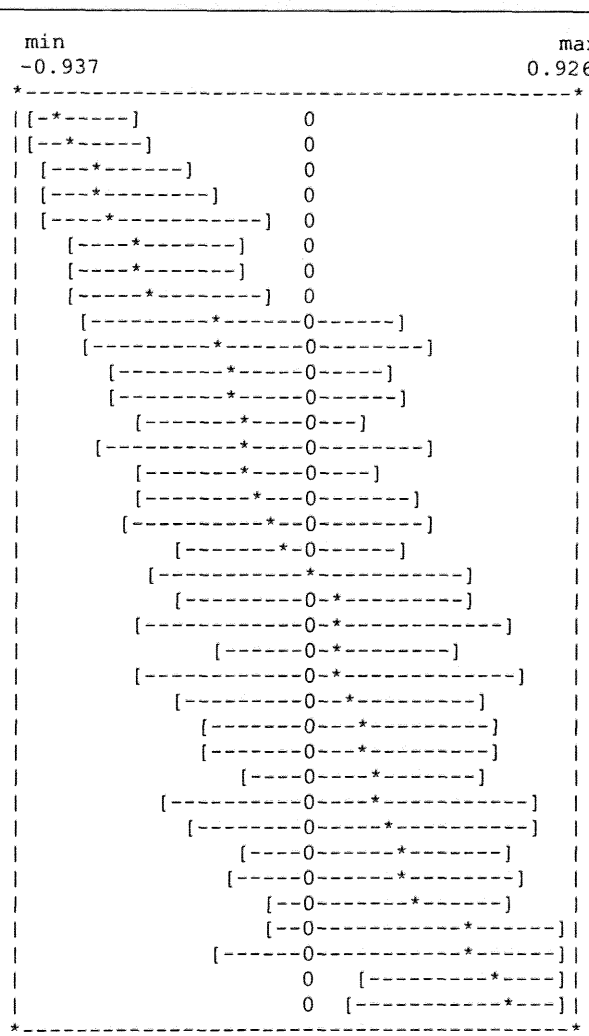
[a] Confidence limit
[b] Pr > F

5.5.1.2 Meta-analyses of rice and wheat productivity trends

The results from the RRCA were compared with those from the meta-analysis using correlation coefficients as effect-size estimates. For rice, the combined effect-size estimate obtained from meta-analysis of data from 33 LTE was −0.15 with 95% CL of −0.239 to −0.056 (Table 5.3). The CL does not include the value

Table 5.3 Dot plots of correlation coefficients between rice yield and cropping years, their 95% confidence limits, and the estimated effect-size estimate (r) in 33 rice-wheat LTE[a]

Site	Lower 95% CL[b]	r	Upper 95% CL
1 Ludhiana1	-0.94	-0.83	-0.58
2 Pantnagar4	-0.93	-0.81	-0.51
3 Pantnagar1	-0.88	-0.72	-0.40
4 Pusa	-0.90	-0.71	-0.29
5 Bhairahwa2	-0.91	-0.68	-0.14
6 Bhairahwa1E	-0.81	-0.58	-0.20
7 Tarahara	-0.81	-0.58	-0.20
8 Karnal	-0.80	-0.55	-0.14
9 Palampur1	-0.75	-0.31	0.32
10SichuanL	-0.78	-0.29	0.42
11Faizabad	-0.68	-0.26	0.29
12Palampur2	-0.68	-0.23	0.34
13Rewa	-0.59	-0.23	0.20
14Karnal1	-0.69	-0.20	0.42
15Barrackpore	-0.57	-0.19	0.25
16Pantnagar3	-0.58	-0.14	0.36
17Kalyani	-0.62	-0.14	0.42
18Nepalgunj	-0.45	-0.06	0.35
19Ludhiana4	-0.52	0.04	0.58
20Varanasi	-0.44	0.09	0.58
21Ludhiana3	-0.56	0.11	0.69
22Pantnagar2	-0.32	0.11	0.50
23Dinajpur2	-0.59	0.12	0.73
24Sabour	-0.43	0.13	0.62
25Jabalpur	-0.34	0.21	0.65
26Nangong	-0.36	0.21	0.67
27Parwanipur	-0.22	0.23	0.60
28Dinajpur1	-0.50	0.24	0.78
29SichuanE	-0.41	0.29	0.78
30Pura	-0.22	0.33	0.72
31Navsari	-0.24	0.36	0.76
32Bhairahwa1L	-0.10	0.36	0.69
33Jiangsu	-0.10	0.57	0.88
34Ludhiana2	-0.32	0.57	0.93
35Kanpur	0.21	0.65	0.87
36Raipur	0.14	0.68	0.91



Meta-analysis (WAVGMETA):

Combined			
All sites	**-0.239**	**-0.150**	**-0.056**
IGP	**-0.339**	**-0.244**	**-0.135**
Non-IGP	**-0.064**	**0.119**	**0.294**

[a] For details of LTE, see Ladha et al. (2003). Some LTE had 2 crops of rice per year, hence 36 correlation coefficients are given from 33 LTE. Bhairahwa1, Dinajpur, and Sichuan followed the rice-rice-wheat system. The letter E after a site name denotes early rice and L late rice. Sites with more than one LTE are distinguished by the numbers following the site names.

[b] CL, confidence limit

zero suggesting the significant negative correlation between rice yield and cropping years. In short, there was a significant overall decline in rice yield with

continuous cropping. In wheat, the combined effect size estimate (r) for 33 LTE was 0.02 with a 95% confidence limit of −0.076 to 0.119 (Table 5.4). Since the 95% CL includes the value zero, there was no significant correlation between cropping years and wheat yield.

Table 5.4 Dot plots of correlation coefficients between wheat yield and cropping years, their 95% confidence limits, and the estimated effect-size estimate (r) in 33 rice-wheat LTE[a]

Site	Lower 95% CL[b]	r	Upper 95% CL	min −0.893 … max 0.967
1 Navsari	-0.89	-0.67	-0.20	[----*---------] 0
2 Pantnagar4	-0.84	-0.54	0.01	[------*----------]
3 Bhairahwa2	-0.86	-0.53	0.10	[------*----------0-]
4 Tarahara	-0.75	-0.46	-0.02	[------*--------]0
5 Sichuan	-0.82	-0.40	0.30	[--------*--------0-----]
6 Pusa	-0.77	-0.40	0.17	[-------*--------0--]
7 Pantnagar3	-0.73	-0.38	0.13	[------*--------0-]
8 Nangong	-0.76	-0.35	0.25	[--------*-------0----]
9 Jabalpur	-0.73	-0.35	0.20	[-------*-------0---]
10Kanpur	-0.73	-0.34	0.21	[-------*-------0---]
11Bhairahwa1	-0.64	-0.28	0.19	[-------*-----0---]
12Parwanipur	-0.61	-0.22	0.24	[--------*----0----]
13Pura	-0.65	-0.21	0.34	[---------*----0------]
14Palampur2	-0.67	-0.16	0.46	[----------*---0---------]
15Varanasi	-0.61	-0.15	0.39	[---------*---0-------]
16Faizabad	-0.57	-0.08	0.45	[----------*-0---------]
17Barrackpore	-0.38	0.02	0.42	[--------*--------]
18Karnal	-0.38	0.08	0.50	[--------0*---------]
19Nepalgunj	-0.31	0.16	0.56	[------0--*--------]
20Kalyani	-0.41	0.16	0.64	[--------0--*----------]
21Raipur	-0.47	0.18	0.71	[----------0---*----------]
22Ludhiana1	-0.31	0.20	0.62	[------0---*--------]
23Jiangsu	-0.43	0.23	0.73	[---------0----*----------]
24Pantnagar1	-0.18	0.29	0.65	[---0-----*-------]
25Ludhiana2	-0.57	0.32	0.86	[------------0------*-----------]
26Ludhiana4	-0.23	0.36	0.76	[----0-------*-------]
27Rewa	-0.01	0.40	0.70	[0-------*------]
28Sabour	-0.14	0.42	0.78	[--0--------*-------]
29Karnal1	-0.02	0.56	0.86	[0-----------*------]
30Dinajpur1	-0.16	0.57	0.89	[---0-----------*------]
31Pantnagar2	0.25	0.60	0.81	0 [-------*----]
32Palampur1	0.16	0.62	0.86	0 [---------*-----]
33Ludhiana3	0.51	0.86	0.97	0 [-------*]
Meta-analysis (WAVGMETA):				
Combined	**-0.076**	**0.022**	**0.119**	

[a] For details of LTE, see Ladha et al. (2003). Sites with more than one LTE are distinguished by the numbers following the site names. The letter E after a site name denotes early rice and L late rice.
[b] CL, confidence limit

In the IGP (sites 1-8, 11, 14-24, 27, 28, 32, 34, and 35), the combined effect-size estimate for rice was higher (-0.244, 95 % CL = -0.339 to -0.135) compared with the overall estimate indicating a stronger negative correlation between cropping years and rice yield (Table 5.3). In the non-IGP, the effect-size estimate was 0.119 with CL ranging from −0.064 to 0.294 (Table 5.3) indicating that

cropping years had no significant effect on rice yield in this group. In wheat, no significant correlation was observed between cropping years and yield in both the IGP and the non-IGP (data not shown).

The dot plots illustrate how the effect-size estimates vary in the different LTE and the extent of negative or positive correlations as shown by the 95% CL (Tables 5.3 - 5.4). Although the rice yield trends were significantly negative in only 8 LTE (sites 1-8), the aggregate (from 33 LTE) showed a significant negative trend (−0.150, 95% CL = −0.239 to −0.056) (Table 5.3). The IGP group showed a stronger negative trend since all of the 8 sites that exhibited significant negative trends were in that group (Table 5.3). Results from the RRCA and meta-analysis are in agreement in that both analyses showed that the trends were significantly negative. However, the combined correlation coefficient obtained from meta-analysis only shows the strength of association between grain yield and cropping years but not the magnitude of yield change unlike the RRCA which gives both the magnitude and significance of the overall yield change.

5.5.2 Productivity trends in rice-rice systems

5.5.2.1 Integrating rice yield trends using randomized regression coefficient analysis

A quantitative evaluation of the aggregate yield trend in rice-rice systems for the dry and wet seasons was done using data from 23 LTE. For both dry and wet seasons, the average yield change was significantly negative (−30.1, 95% CL = −59.0 to −1.1 kg ha^{-1} yr^{-1} during the dry season and −48.1, 95% CL = −65.9 to −30.3 kg ha^{-1} yr^{-1} during the wet season). More intensive rice cultivation has been done at IRRI as compared with the other Asian sites. Thus the sites were grouped into IRRI and non-IRRI in determining the yield trends. There was a significant year x group interaction when the sites were grouped into IRRI and non-IRRI sites during the dry but not the wet season. During the dry season, yield declined significantly in the IRRI sites (−116.5, 95% CL = −162.4 to −70.5 kg ha^{-1} yr^{-1}) but not in the non-IRRI sites. However, during the wet season, yields declined significantly in both IRRI and non-IRRI sites (−66.9, 95% CL = −96.1 to −37.7 kg ha^{-1} yr^{-1} in IRRI sites and −38.4, 95% CL = -59.0 to −17.7 kg ha^{-1} yr^{-1} in non-IRRI sites) and the average trends were not significantly different from each other

as shown by the 95% confidence intervals (Table 5.5). When the trends were considered at the system level, year x group interaction was significant. Yield

Table 5.5 Random regression coefficient analysis of rice yield trends in 23 rice-rice LTE performed using the SAS procedure MIXED for linear mixed models

Sites	No. of data sets	Slope kg ha^{-1} yr^{-1}	Pr > |t|	Lower 95% CL[c] kg ha^{-1} yr^{-1}	Upper 95% CL kg ha^{-1} yr^{-1}
Dry season					
All sites	23	−30.1	0.04	−59.0	−1.08
Yr * region			0.002[d]		
IRRI	3	−116.5	0.001	−162.4	−70.5
Non-IRRI[a]	20	−15.0	0.18	−38.0	7.9
Wet season					
All sites	18	−48.1	0.0001	−65.9	−30.3
Yr * region			0.11[d]		
IRRI[b]	4	−66.9	0.0004	−96.1	−37.7
Non-IRRI[a]	14	−38.4	0.001	−59.1	−17.7
System					
All sites	18	−77.4	0.0008	−117.4	−37.5
Yr * region			0.0048[d]		
IRRI	4	−170.1	0.0001	−234.8	−105.3
Non-IRRI[a]	14	−52.8	0.006	−88.7	−16.9

[a] Two-season rice crops for 3 LTE in China (6 data sets) are included here because the distinction between wet and dry season crop is less clear than in tropical regions. Two other experiments have no wet season rice and two experiments had no dry season rice,
[b]The IRRI-LTCEE included 2 wet season crops (early and late).
[c] Confidence limit
[d] Pr > F

trends declined significantly in both IRRI (−170.1, 95% CL = −234.2 to −105.3 kg ha^{-1} yr^{-1}) and non-IRRI sites (−52.8, 95% CL = −88.7 to −16.9 kg ha^{-1} yr^{-1}) but the magnitude of yield decline was a significantly greater in the IRRI than in the non-IRRI sites (Table 5.5).

5.5.2.2 Meta-analyses of yield trends in rice-rice systems in different Asian countries

Dot plots of correlation coefficients of rice yield vs. cropping years in tables 5.6 and 5.7 illustrate the variation in yield trends in LTE at IRRI and other Asian sites during the dry and wet seasons. During the dry season, all IRRI sites had

Table 5.6 Dot plots of correlation coefficients between rice yield and cropping years, their 95% confidence limits, and the estimated effect-size estimate (r) in 23 rice-rice LTE during the dry season[a]

Site	Lower 95% CL[b]	r	Upper 95% CL	min -0.907 ... max 0.976
1 IRLTCC	-0.91	-0.79	-0.56	|[--*----] 0 |
2 NresIR	-0.90	-0.76	-0.49	|[--*-----] 0 |
3 ShipaiE	-0.89	-0.66	-0.13	|[----*-----------] 0 |
4 IRLTE	-0.83	-0.63	-0.31	| [---*------] 0 |
5 FujianE	-0.87	-0.57	0.05	| [-----*-----------0] |
6 GazBD	-0.81	-0.47	0.11	| [------*----------0-] |
7 JiangxiE	-0.77	-0.45	0.04	| [------*---------0] |
8 Bicol	-0.65	-0.33	0.10	| [------*------0-] |
9 NresVi	-0.69	-0.28	0.27	| [--------*-----0-----] |
10GazD	-0.70	-0.27	0.30	| [---------*-----0-----] |
11NresBi	-0.62	-0.25	0.20	| [-------*-----0---] |
12Bhubane	-0.61	-0.24	0.23	| [--------*----0----] |
13ShipaiL	-0.70	-0.21	0.42	| [---------*----0--------] |
14JiangxiL	-0.59	-0.15	0.36	| [--------*---0------] |
15FujianL	-0.61	-0.05	0.54	| [-----------*0----------] |
16Mardim	-0.60	0.00	0.60	| [------------*------------] |
17Maros	-0.55	0.03	0.60	| [-----------*------------] |
18NresPR	-0.33	0.11	0.51	| [------0-*--------] |
19Sukam	-0.38	0.12	0.57	| [--------0-*---------] |
20ComB	-0.47	0.18	0.70	| [----------0---*----------] |
21PhRLTE	-0.16	0.27	0.61	| [---0----*-------] |
22Lanra	-0.33	0.27	0.71	| [-------0----*---------] |
23CLRRI	0.72	0.92	0.98	| 0 [----@|
Meta-analysis (WAVGMETA):				
Combined				
All	**-0.355**	**-0.259**	**-0.146**	
IRRI	**-0.830**	**-0.932**	**-0.589**	
Non-IRR	**-0.219**	**-0.098**	**0.027**	

[a] For details of LTE, see Dawe et al. (2000). E after a site name denotes early rice and L late rice.
[b] CL, confidence limit

significant negative trends (sites 1, 2 and 4); and during the wet season, all IRRI sites (sites 2, 4 and 5) except for site 10 had significant negative trends. Among the non-IRRI sites, only Shipai (site 3) in China showed a significant negative trend during the dry season while CLRRI (site 1) in Vietnam and PhilRice in the Philippines (site 3) showed significant negative trends during the wet season. However, the combined correlation computed by the SAS macro WAVGMETA showed an overall negative correlation coefficient of -0.259 (95% CL = -0.355 to -0.146) during the dry- and -0.387 (95% CL = −0.469 to −0.260) during the wet season indicating an overall yield decline (Tables 5.6 and 5.7). When only the

IRRI sites were analyzed, a stronger negative correlation between yield and cropping years was obtained (−0.830, 95% CL = −0.932 and −0.589 during the dry season, and −0.559, 95% CL = −0.654 and −0.324 during the wet season). For

Table 5.7 Dot plots of correlation coefficients between rice yield and cropping years, their 95% confidence limits and the estimated effect-size estimate (r) in 18 rice-rice LTE during the wet season[a]

Site	lower 95% CL[b]	r	upper 95% CL	min −0.917 … max 0.722
1 CLRRIWS	-0.92	-0.71	-0.18	[----*----------] 0
2 IRLTCCEW	-0.83	-0.63	-0.31	[----*-------] 0
3 PhRLTEWS	-0.81	-0.60	-0.23	[----*--------] 0
4 NresIRWS	-0.81	-0.56	-0.14	[-----*---------] 0
5 IRLTCCLW	-0.76	-0.51	-0.12	[-----*---------] 0
6 GazW	-0.80	-0.49	0.03	[-------*----------0]
7 LuisWS	-0.84	-0.43	0.27	[---------*----------0------]
8 Bhubane	-0.66	-0.33	0.12	[--------*-------0--]
9 Mardioff	-0.79	-0.33	0.38	[----------*-------0--------]
10IRLTEWS	-0.63	-0.30	0.13	[-------*-------0--]
11NresPRWS	-0.62	-0.26	0.19	[--------*------0----]
12SukamW	-0.66	-0.26	0.25	[---------*------0-----]
13NresViWS	-0.60	-0.23	0.22	[--------*-----0-----]
14BicLTEWS	-0.57	-0.21	0.22	[--------*----0----]
15NresBiWS	-0.57	-0.19	0.27	[--------*----0------]
16ComTW	-0.63	-0.06	0.56	[--------------*0-------------]
17GazTW	-0.55	0.00	0.55	[-------------*-------------]
18TanayWet	-0.51	0.17	0.72	[------------0---*------------]
Meta-analysis (WAVGMETA):				
Combined				
All	**-0.469**	**-0.387**	**-0.260**	
IRRI	**-0.654**	**-0.559**	**-0.324**	
Non-IRRI	**-0.430**	**-0.315**	**-0.169**	

[a] For details of LTE, see Dawe et al. (2000). E after a site name denotes early rice and L late rice.
[b] CL, confidence limit

non-IRRI sites, the combined correlation coefficient was not significant during the dryseason indicating more stable rice yields (Table 5.6) as was also concluded by Dawe et al. (2000). Nevertheless, non-IRRI sites showed a significantly negative trend during the wet season (−0.315, 95% CL = −0.430 and −0.169).

Results from meta-analysis are in agreement with RRCA in that non-IRRI sites showed significant negative trends during the wet season but not during the dry season. Both analyses also showed that system yield trends were significantly negative in both the IRRI and non-IRRI sites but RRCA further showed that the decline was less in magnitude in the non-IRRI sites than in the IRRI sites.

5.5.3 Effect of organic matter treatments in combination with inorganic fertilizers on yield trends in rice-wheat systems

5.5.3.1. Random regression coefficient analyses

Nineteen of the rice-wheat LTE had treatments with farmyard manure (FYM) combined with NPK fertilizers in addition to the treatment with only inorganic NPK which had been used for analyzing yield trends. These LTE were of different duration (Table 5.8). By including a year x treatment interaction in the analysis, we were able to compare the aggregate yield trend of treatments with100% NPK, and that with NPK+FYM. There was a significant year x treatment interaction in rice but not in wheat yield. The aggregate rice yield change was significantly positive (34.7, 95% CL = 6.3 to 63.1 kg ha^{-1} yr^{-1}) with FYM. On the other hand, the aggregate yield change with 100% NPK treatment was not significantly different from 0. However, the 95% CL indicate that the rice yield trends (slopes) did not vary significantly between the two treatments. In wheat there was no significant yield change with and without FYM (Table 5.9).

Table 5.8 Duration of LTE with FYM treatment in various South Asian sites.

LTE site	Duration (years)
Ludhiana1	16
Bhairahawa1	21
Tarahara	23
Faizabad	15
Palampur2	15
Pantnagar3	17
Kalyani	14
Napalgunj	24
Ludhiana4	11
Varanasi	15
Dinajpur	9
Sabour	16
Jabalpur	15
Parwanipur	21
Pura	15
Navsari	13
Ludhiana2	7
Kanpur	15
Raipur	11

Table 5.9 Random coefficient regression analysis of rice and wheat yield trends in treatments with 100% NPK fertilizer and FYM treatment in combination with inorganic NPK fertilizer performed using the SAS procedure MIXED for linear mixed models.

Treatment	Slope kg ha^{-1} yr^{-1}	Pr > |t|	Lower 95% CL[a] kg ha^{-1} yr^{-1}	Upper 95% CL[a] kg ha^{-1} yr^{-1}
Rice				
Yr * treatment		0.01[b]		
100% NPK	4.5	0.75	−23.8	32.8
FYM+NPK	34.7	0.02	6.3	63.1
Wheat				
Yr * treatment		0.09[b]		
100% NPK	8.0	0.60	−22.9	38.8
FYM+NPK	-11.3	0.45	−42.1	19.1

[a] Confidence limit
[b] Pr > F

Fig. 5.1 shows a bar graph of rice yield averages obtained from 13-19 LTE during the 1st to the 15th year in treatments with and without FYM. All the LTE ran for at least 7 years but only 13 LTE ran for 15 or more years. From years 1 to 11, the average rice yield was higher without FYM but the difference between the two treatments appeared to decrease with time. However, mean comparison by the paired t-test for each year showed that the difference between the two treatments was significant only in years 1, 2 and 6 (analysis not shown).

The regression lines of rice yields over time with and without FYM were drawn based on the slope and intercept obtained from RRCA (Fig. 5.2). These trend lines suggest that a yield increase from FYM may be observed only after 15 years. If the linear trend were extended beyond 15 years, the treatment with FYM would show a higher yield than that without FYM. However, of 19 LTE, only 4 ran for more than 20 years. The longest duration LTE is at Nepalgunj (24 years) followed by Tarahara (23 years). In these two LTE the increase in rice yield with FYM is evident. Fig. 5.3 shows that in Nepalgunj, rice yields with FYM had been consistently higher than yields without FYM starting from the 19th year. In

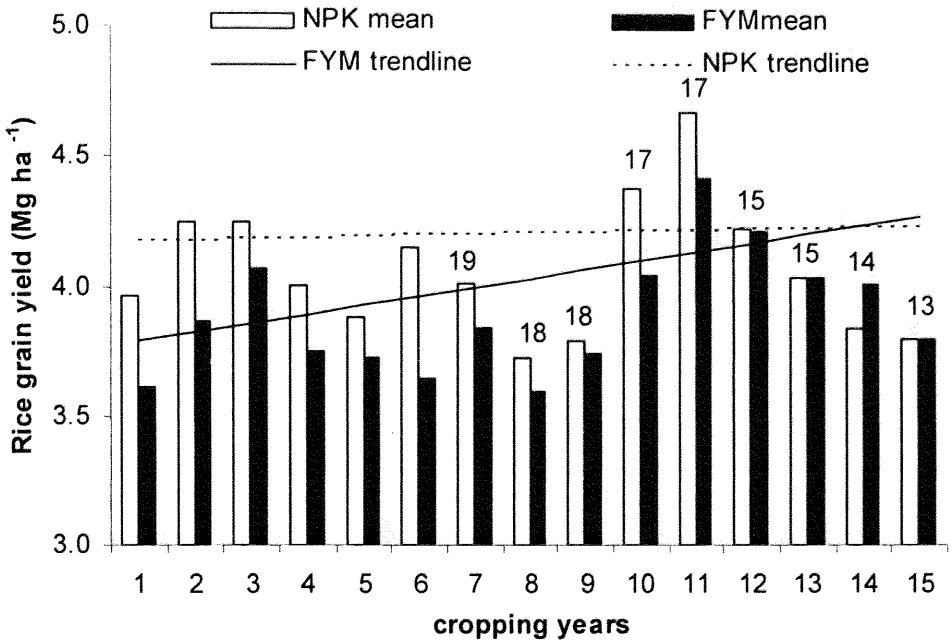


Fig. 5.1 Average yields of rice obtained from 13-19 LTE for the 1st to 15th crop in treatments with NPK alone and NPK in combination with FYM. Trend lines were drawn using the slopes and intercepts determined by the RRCA. Numbers above the bars indicate the number of LTE used in measuring the average yield. The number of LTE used from year 1 to 7 is 19.

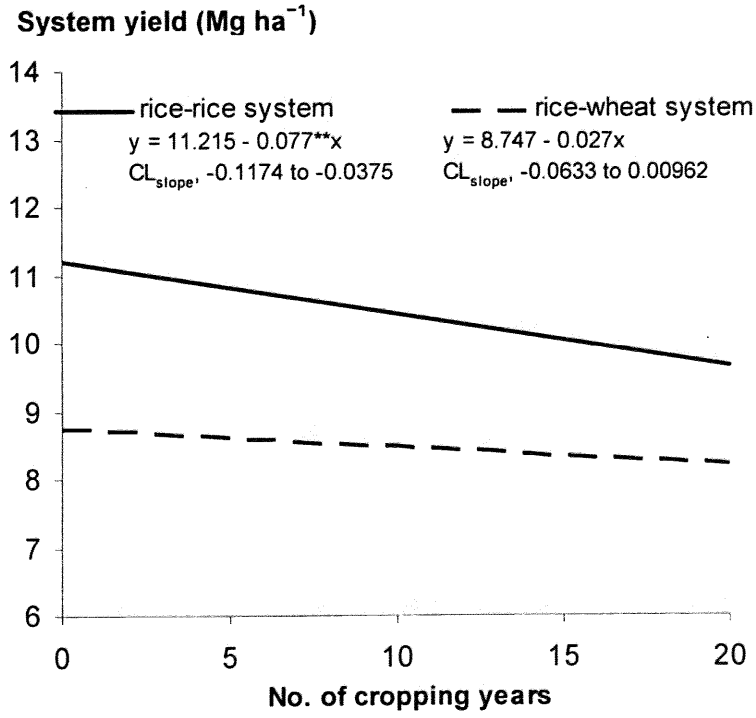


Fig. 5.2 Aggregate system yield trends in rice-rice and rice-wheat systems from 18 and 33 LTE, respectively, across Asia.

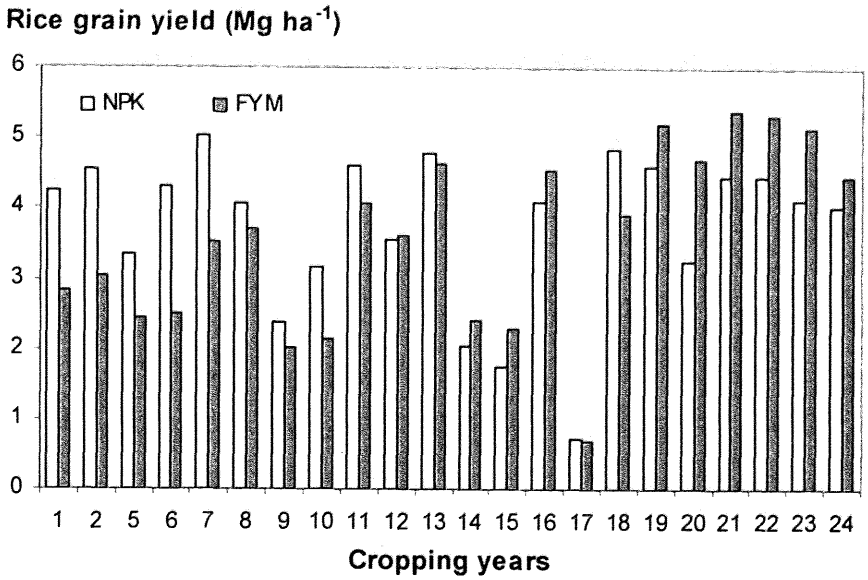


Fig. 5.3 Rice yields with 100% NPK and NPK+FYM treatment in the rice-wheat LTE at Nepalgunj.

Tarahara, the average yield from the 20th to the 23rd year with FYM was higher at 3.4 Mg ha^{-1} than that without FYM (2.7 Mg ha^{-1}).

The above results indicate that addition of FYM did affect the rice yield trend as shown by the significant year x treatment (NPK vs. NPK+FYM) interaction and the significant positive yield trend obtained in the FYM+NPK treatment which was not observed in the NPK treatment. However, the present results do not provide sufficient evidence on the increase in rice yield due to FYM in the long term (beyond 15 years) since most of the LTE ran for only 15 years at most. Only 2 LTE, which ran for more than 20 years have demonstrated this effect. Moreover, since most of the LTE ran for only 15 years we can only conclude that there was a positive linear trend up to 15 years at least. There is a need to analyze subsequent data to determine the trend beyond 15 years. Yields with FYM would tend to plateau at some point just as SOM formation and decomposition is expected to reach a "steady state" after a long period under the same soil management.

5.5.3.2 Meta-analyses

In agreement with the RRCA, the combined correlation between cropping years and rice grain yield from 19 LTE treated with NPK + FYM was 0.196 with a 95% CL of 0.073 to 0.308 indicating that the trends were significantly positive. On the other hand, the combined correlation coefficient obtained from treatments without FYM (−0.025)was not significantly different from zero with confidence

limits of −0.146 and 0.097 (Table 5.10). However, the 95% CL indicate that the combined correlation coefficients obtained with and without FYM were not significantly different from each other. Dot plots of correlation coefficients with their 95% confidence limits in each site in treatments with and without FYM are given in Table 5.10.

5.6 Discussion

A simple linear regression approach normally used to analyze yield trends does not allow combined or integrative analyses and interpretations. Instead, the number of data sets with positive or negative yield trends are used to make assessments on the global scenario. In this paper, we illustrated how yield trends from several sites across Asia can be integrated to assess the magnitude and significance of yield change in Asia and make regional or global interpretations using the RRCA and meta-analyses.

The combined analyses showed a significantly negative rice yield trend thereby suggesting widespread yield decline in both rice-rice and rice-wheat systems of Asia. In the rice-rice system, the magnitude of rice yield decline was greater in the IRRI sites than in the non-IRRI sites during the dry season as was also reported earlier (Dawe et al., 2000). However, yield trends did not vary significantly between IRRI and non-IRRI sites during the wet season. Moreover, system yield trend was significantly negative in both the IRRI and non-IRRI sites although the magnitude of yield decline in the IRRI sites was significantly greater than in the non-IRRI sites. These results imply that the extent of yield decline in rice-rice systems is a matter of concern not only at IRRI.

Unlike for rice-rice, the system productivity of rice-wheat had no change as described earlier. The stable yield trends in wheat contributed to the sustainability of the rice-wheat system although total crop productivity of rice-rice is about 15% higher than the rice-wheat system (Fig.2). Nevertheless, rice yields had a widespread decline in both systems.

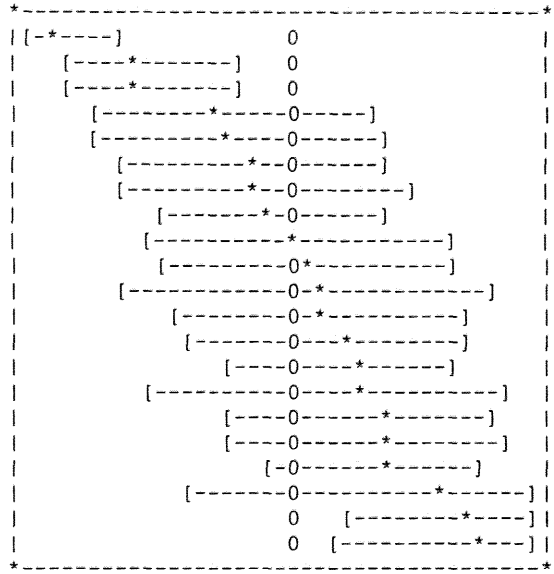
Interpretations of yield trends in the rice-wheat system using RRCA and meta-analysis were consistent with those made earlier by simple linear regression analysis, that is, rice yields declined significantly but wheat yields remained stable (Ladha et al., 2003). The present combined analysis showed that the combination of inorganic (NPK) and an organic source (FYM) can somehow mitigate the yield decline of rice in the rice-wheat system although more yield data from longer duration LTE are needed to confirm and quantify yield increases due to FYM in the long-term. Earlier analyses, based on a

comparison of individual yield trends in each location and count of the number of LTE with significant *p*-values at a given location (Dawe et al., 2003), do not agree with this.

Table 5.10 Dot plots of correlation coefficients between rice yield and cropping years, their 95% confidence limits, and the estimated effect-size estimate (r) in treatments with 100% NPK and NPK+FYM.

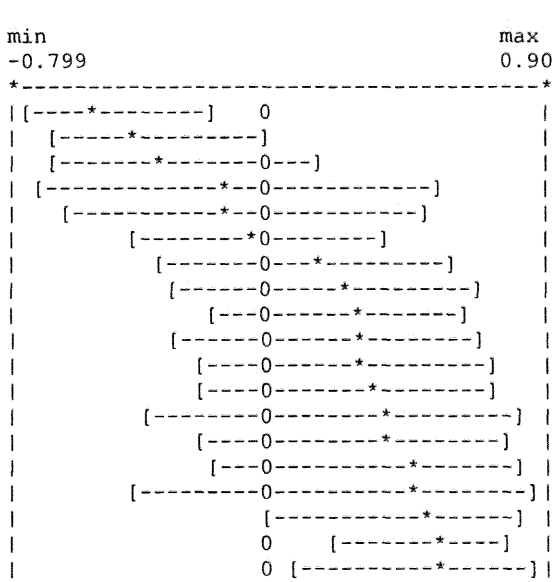
100%NPK

Site[a]	Lower 95% CL[b]	r	Upper 95% CL
1 Ludhiana1	-0.94	-0.83	-0.59
2 Bhairahawa1E	-0.81	-0.58	-0.19
3 Tarahara	-0.81	-0.58	-0.19
4 Faizab	-0.68	-0.26	0.29
5 Palampur2	-0.68	-0.24	0.34
6 Pantnagar3	-0.58	-0.14	0.36
7 Kalyani	-0.62	-0.14	0.43
8 Nepalgunj	-0.45	-0.06	0.35
9 Ludhiana4	-0.52	0.04	0.58
10Varanasi	-0.45	0.08	0.57
11Dinajpur2	-0.59	0.12	0.73
12Sabour	-0.43	0.13	0.62
13Jabalpur	-0.34	0.21	0.65
14Parwanipur	-0.22	0.23	0.60
15Dinajpur1	-0.50	0.24	0.78
16Pura	-0.22	0.33	0.72
17Navsari	-0.24	0.36	0.76
18Bhairahawa1L	-0.10	0.36	0.69
19Ludhiana2	-0.35	0.55	0.92
20Kanpur	0.21	0.65	0.87
21Raipur	0.14	0.68	0.91
Meta-analysis (WAVGMETA):			
Combined	**-0.146**	**-0.025**	**0.097**



NPK+FYM

Site	Lower 95% CL	r	Upper 95% CL
1 Bhairahawa1E	-0.80	-0.56	-0.17
2 Tarahara	-0.73	-0.43	0.00
3 Ludhiana1	-0.73	-0.37	0.16
4 Dinajpur2	-0.74	-0.15	0.57
5 Ludhiana4	-0.68	-0.13	0.51
6 Bhairahawa1L	-0.47	-0.04	0.40
7 Pantnagar3	-0.35	0.15	0.59
8 Palampur2	-0.33	0.25	0.69
9 Parwanipur	-0.18	0.27	0.63
10Kalyani	-0.30	0.28	0.70
11Faizabad	-0.24	0.31	0.71
12Jabalpur	-0.21	0.34	0.72
13Dinajpur1	-0.40	0.36	0.83
14Navsari	-0.23	0.37	0.77
15Raipur	-0.20	0.46	0.83
16Ludhiana2	-0.44	0.47	0.90
17Varanasi	-0.02	0.49	0.80
18Nepalgunj	0.20	0.56	0.79
19Pura	0.08	0.57	0.84
20Sabour	0.08	0.59	0.85
21Kanpur	0.18	0.64	0.87
Meta-analysis (WAVGMETA):			
Combined	**0.073**	**0.196**	**0.308**



[a] For details of LTE, see Ladha et al. (2003). Sites with more than one LTE are distinguished by the numbers following the site names. The letter E after a site name denotes early rice and L late rice.
[b] CL, confidence limit.

CHAPTER 6

EVALUATING EFFECTS OF ORGANIC AMENDMENTS ON SOIL PARAMETERS AND SOIL QUALITY IN THREE LONG-TERM EXPERIMENTS

6.1 Summary

Long-term experiments provide opportunities to assess changes in soil quality as influenced by soil and fertilizer management. Soil organic matter (SOM), the key attribute of soil quality can be divided into labile or rapidly decomposed, and stable or slowly decomposed fractions. These fractions are descriptive of the quality of soil organic matter. Soil C oxidized by neutral $KMnO_4$, or permanganate-oxidizable C (MnOC), has been used as an index of labile C by several workers, although the nature of organic C oxidized has not been well elucidated. However, results from this study indicate that MnOC is a better indicator of lignin content than labile C and thus it may be used to monitor changes in the stored organic matter or the slow C pool resulting from various agronomic practices. On the other hand hot water-extractable C (HWEC) was used to measure the readily decomposable C. Hot water-extractable C did not correlate with total C across years. This lack of correlation makes it important to monitor HWEC together with total C or MnOC in assessing the change in soil quality over the years. Hot water extractable C was found to correlate well with microbial biomass C (MBC) and potentially-mineralizable N (PMN) across years.

The impacts of continuous applications of an organic source [farmyard manure (FYM), green manure (GM), rice straw (RS), rice straw compost (RSC), and wheat straw, (WS) in combination with inorganic fertilizers (NPK) on soil quality and productivity of rice-wheat systems were investigated in three rice-wheat long-term experiments in Fukuoka, Japan, after 40 years, in Ludhiana, India, after 20 years, and in Bhairahawa, Nepal, after 15 years, by measuring changes in the stable and decomposable soil C and N pools, and soil microbiological parameters relative to unfertilized and inorganically fertilized controls. Organic amendment had positive but variable effects.

In Fukuoka, all the organic residue treatments (RS, RSC, and WS) with or without N brought about significant increases in total C and N, MnOC, HWEC and PMN as compared with the inorganically fertilized and unfertilized controls after 40 years. The highest accumulation of total C (23%) and N (72%) in the soil was from RSC as compared with that from RS (C, 7% and N, 33%) and rye grass / WS (C, 9% and N, 29%).

Incorporation of RSC also increased MBC under both aerobic and flooded conditions. A lower metabolic quotient (qCO_2) was obtained in the RS and RSC treatments as compared with the control under aerobic condition indicating an efficient utilization of C by microorganisms.

In Ludhiana, total pools of C and N, and HWEC were 28-40% higher than the control after 20 years only in the FYM+NPK plots where HWEC was also maintained with time. HWEC showed a declining trend over the years in the other treatments (100% NPK, WS+NPK and GM+NPK) while total C increased by 17% on the average from the 5^{th} to the 20^{th} year. In Bhairahawa, though total organic C and N increased with an organic amendment, HWEC did not. Accumulated percent increases in C and N as fractions of the applied organic fertilizers were 11-23 and 37-39 from FYM, 4-21 and 19-41 from GM, and 3 and 24 from WS respectively. Farmyard manure also improved available P, cation-exchange capacity (CEC), potential mineralizable N (PMN) and dehyrogenase activity, but microbial biomass C (MBC), basal respiration, flush of CO_2 after rewetting dried soil, and qCO_2 remained unchanged.

6.2 Introduction

Developing and implementing management strategies that maintain the quality of soil, are essential to enhance the performance and sustainability of an agroecosystem. Carbon is the key attribute of soil quality (Christensen and Johnston, 1997; Carter, 2002) because it influences nutrient cycling, soil structure, water availability, and other important soil properties (Doran et al., 1998; Arshad and Coen, 1992; Yakovchenko et al., 1998). Increasing soil C has a significant impact on the mineralization and recycling of C and N (Sanchez et al., 2004). Although sustainability issues are much broader than soil quality, the strong emphasis on maintaining the natural-resource base ensures proper ecosystem functioning (Miller and Wali, 1995).

Labile SOM fractions such as the light fraction, macroorganic matter or particulate C (Christensen, 1986; Hussain et al 1999), microbial biomass C (Sparling, 1992; Yoshikawa and Inubushi, 1995;), mineralizable C (Franzluebbers et al., 1994), carbohydrates, and enzymes (Deng and Tabatabai, 1996a; 1996b; 1997) are highly responsive to changes in C inputs to the soil and will provide a measurable change prior to any such change in total organic matter (Gregorich and Janzen, 1996). In contrast, the more stable (humified) pools are probably the more appropriate and representative fractions for C sequestration characterization (Cheng and Kimble, 2001).

Fractionation of soil organic C, based on its susceptibility to oxidation with KMnO4 solutions of various concentrations (33 - 333 mM), was introduced by Loginow et al. (1987) and was modified by Blair et al. (1995) using a single concentration of $KMnO_4$ (333 mM) as the oxidizing agent. Carbon which is oxidized by $KMnO_4$ in 1 h was considered as labile, and the remaining C that is not oxidized, as non-labile. Subsequently, a C management index (CMI) based on changes in the total, labile and non-labile C fractions was developed and several workers (Blair et al., 1997; Blair et al., 2001; Murage et al., 2000; Shrestha et al., 2002; Whitbread et al., 1998) used the method to determine short-term changes in the labile C fraction. However, the nature of soil C directly oxidized by neutral permanganate and the rates of oxidative reaction of organic compounds with $KMnO_4$ have not been well elucidated.

Several researchers have conducted long-term cropping system experiments (LTE) in Asia to evaluate various agronomic and cultural practices. Mostly assessments were made based on crop productivity trends, and sometimes changes in soil total C and various nutrients (Nambiar, 1994; Yadav et al., 1998; 2000; Dawe et al., 2000; Duxbury et al., 2000; Tsuchiya, 2000, Bhandari et al., 2002; Regmi et al., 2002). However, equally important is to study the decomposable C and N fractions which take active part in various soil transformation processes in response to agronomic interventions (Schulz and Korschens, 1998). Two LTE with 15-20 year rice-wheat rotation in India and Nepal having various combinations of inorganic and organic sources of nutrients were utilized to extend earlier research (Bhandari et al., 2002; Regmi et al., 2002).

6.3 Objectives

The objectives of this study were to determine:

a. the reactivity of diverse soil organic compounds with $KMnO_4$ in order to judge the reliability of MnOC as an index of labile C;
b. the cumulative effects of combinations of inorganic and organic sources on various chemical and microbiological soil properties with emphasis on decomposable C and N fractions in 3 long-term experiments in Japan, India, and Nepal;
c. the time trends of a few key soil parameters associated with C and N dynamics in India and Nepal; and
d. the interrelationships among some of the key soil quality parameters.

6.4 Materials and Methods

6.4.1 Assessing the Reliability of Permanganate-oxidizable C as an Index of Soil Labile C

6.4.1.1 Organic Materials and Soils Used

Laboratory-grade sugars, amino acids, and other organic acids (with purity ≥ 98%) were obtained from Sigma-Aldrich. Pyrogallol, ACS was obtained from Fisher Scientific and cellulose, microcrystalline (Avicel®) was from Merck. Dried farmyard manure [cow (Bus indicus) dung] was obtained from the Bhairahawa experiment station in Nepal, while the wheat (Triticum aestivum L.) straw came from an experimental field in Parwanipur, also in Nepal. Rice (Oryza sativa L.) straw together with Azolla microphilla and Sesbania rostrata were taken from a long-term biofertilizer experiment at the International Rice Research Institute IRRI. The farmyard manure was air-dried while the plant materials were oven-dried at 70°C. All materials were finely ground to 0.5 mm.

Soils used for paddy rice production of varying texture and total C and N contents were from farmers' fields and a long-term experiment at the IRRI farm in the Philippines and rice-wheat soils from 2 long-term experiments in Ludhiana and Palampur in India, involving different organic matter treatments. Their physico-chemical properties are given in Table 6.1. All soil samples were air-dried and ground to pass through a 2 mm sieve. For total C, N, and MnOC, soils were ground to pass through a 0.5 mm sieve.

The double-cropped rice long-term experiment at the experimental farm of IRRI, Philippines, began in 1985 with inorganic (urea) and organic (Azolla microphylla Lam. and Sesbania rostrata Bremek. Oberm.) fertilizer treatments. Details of this experiment were reported in Ladha et al. (2000). Soils sampled from the 0-20 cm layer in the 12th and 14th yr after the wet-season crop (25th and 29th crop) from the treated and control plots were analyzed for total and labile C.

The rice-wheat long-term experiment at the experimental farm of the Punjab Agricultural University, Ludhiana, India, included treatments with different combinations of inorganic and organic fertilizers for rice: 0 NPK (control), 100% of the recommended NPK, 50% recommended NPK + 6 Mg ha-1 farmyard manure, 50% recommended NPK + 50% N in green manure (Sesbania cannabina Linu & Merrill), and 75% recommended N + 50% PK + 6 Mg ha-1 wheat cut straw. Details of this experiment were reported in Bhandari et al. (2002).

Table 6.1 Physico-chemical characteristics of rice soils used in this study.

Site	Soil classification	pH	Carbon		Total N	Clay	Silt	Sand
			Total	Organic	g kg^{-1}			
Philippines								
Bugallon	Typic Haplaquoll	6.3	22.5	19.8	2.01	300	500	200
IRRI Farm	Andaqueptic Haplaquoll	6.6	14.2	12.5	1.22	620	310	70
Gapan	Aeric Tropaquept	6.6	14.6	11.5	1.41	230	440	330
Luisiana	Aquic Troporthent	4.8	17.5	14.1	1.69	560	400	40
Maahas	Andaqueptic Haplaquoll	5.9	20.8	18.6	1.75	430	440	130
Maligaya	Ustic epiaquerts	6.3	20.2	14.7	1.76	410	500	90
Pila	Psammenthic Anthraquent	7.3	36.2	25.9	3.64	370	340	290
Urdaneta	Aeric Tropaquept	6.5	11.1	10.3	1.13	300	550	150
Bay	Aeric Vertic Epiaqualf	6.5	54.4	49.6	4.54	450	440	110
Pangil	Aeric Tropaquept	5.7	35.4	31.8	3.14	590	380	30
India								
Ludhiana	Typic Ustochrept	8.2	4.57	4.35	0.80	70	40	890
Palampur	Typic Hapludalf	5.6	16.0	14.5	2.50	300	540	160

6.4.1.2 Determination of MnOC [Modified from Blair et al. (1995)]

Standardization of KMnO4 Solutions

A 33 *m*M solution of $KMnO_4$ was prepared by dissolving 5.2 g of $KMnO_4$ crystals in 1000 ml of deionized-distilled water. Low heat was applied to completely dissolve the crystals. The $KMnO_4$ solution was stored in an amber bottle and is stable for up to 1 month. The exact concentration of $KMnO_4$ solution was determined by titration against 0.0500 g As_2O_3, dissolved in 2 ml of 20% NaOH. The solution was acidified with 2 ml of concentrated HCl and treated with 4 drops of 0.25 *m*M KI prior to titration with 33 mM $KMnO_4$. Avery small amount of KI is added as a catalyst. Without a suitable catalyst, Mn^{7+} is reduced only to an average state between Mn^{3+} and Mn^{4+} instead of Mn^{2+} (Willard et al., 1956). A digital buret (Digitrate™, Jencons Scientific Ltd., UK) that could measure up to 0.01 ml was used to dispense the $KMnO_4$. A faint pink color, which

persisted for about 30 seconds, was considered as the end point. The reactions involved in the titration are as follows:

$As_2O_3 + 4\ OH^- = 2HAsO_3^{2-} + H_2O$

$5\ H_3AsO_3 + 2MnO_4^- + 6H^+ = 5\ H_3AsO_4 + 2Mn^{2+} + 3\ H_2O$

$10I^- + 2MnO_4^- + 16H^+ = 5I_2 + 2Mn^{2+} + 8\ H_2O$

Thus for every 5 moles of As_2O_3, 4 moles of MnO_4^- is reduced. The exact concentration of $KMnO_4$ was calculated using the following equation:

$$mM\ KMnO_4 = [wt.\ of\ As_2O_3\ (g) \times 1000\ (ml/l)] / [vol.\ KMnO_4\ (ml) \times 197.84 \times 5/4 \times 0.001] \quad [1]$$

where:

197.84 = molecular weight of As_2O_3 (g/mole)

5/4 = 5 moles As_2O_3 / 4 moles MnO_4^- g As_2O_3 / mmole MnO_4^-

0.001 = moles/mmole

Aliquots of 0.6, 1.0, 1.4, 1.8, and 2.0 ml KMnO4 were measured using an accurate volume dispenser (Pipetman™, Gilson Medical Electronics, France) in a 50-ml volumetric flask and diluted to volume. The concentrations of the diluted $KMnO_4$ solutions were computed as follows:

Aliquot (ml) x exact concentration of $KMnO_4$ (mM) / 50 ml [2]

The absorbance of the diluted $KMnO_4$ standards was then measured at 565 nm using a spectrophotometer (Beckman DU® 650, Beckman Coulter Inc. Fullerton, CA), which was adjusted to zero absorbance using deionized-distilled water. The concentration of $KMnO_4$ was plotted against absorbance at 565 nm to obtain a standard calibration curve. The calibration curve was found to be highly reproducible (Fig. 6.1). Thus the concentration of $KMnO_4$ solutions can be quickly determined from the absorbance reading at 565 nm using the calibration curve.

Analysis of Permanganate-Oxidizable C in Pure Organic Compounds

Triplicate samples of pure organic compounds (sugars, amino acids, organic acids, pyrogallol and cellulose) containing 5 mg C, and triplicate samples of 25 mg cellulose were placed in 50-ml centrifuge tubes provided with caps. These

samples were arranged in a randomized complete block design (i.e. samples were grouped by rep and the organic materials randomized within each rep). Samples

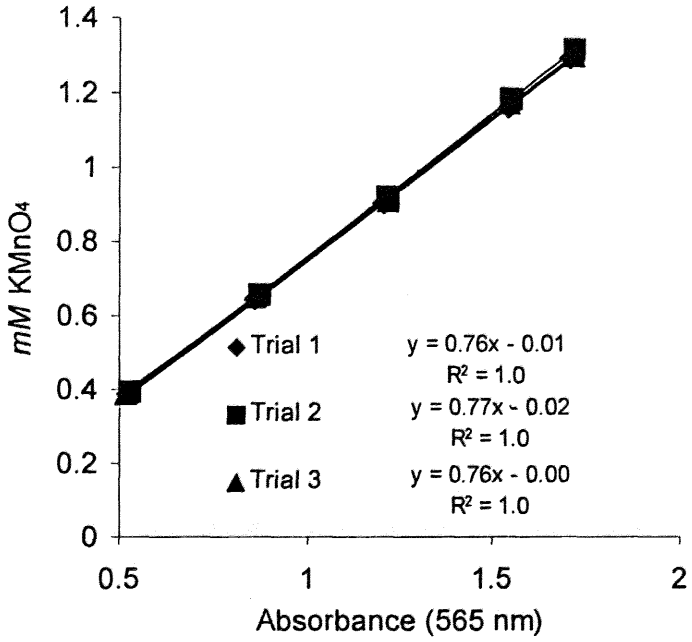


Fig. 6.1 Standard $KMnO_4$ calibration curves ($KMnO_4$ concentration vs. absorbance at 565 nm).

were analyzed replicate-wise (i.e. all rep 1 first followed by rep 2 and rep 3). Five sets of these samples were prepared to measure MnOC at 5 different time intervals (1 min, 1, 3, 6 and 24 h). Using a digital buret (Digitrate™), 25 ml of 33 mM $KMnO_4$ was dispensed into each sample in the centrifuge tubes. The same volume of $KMnO_4$ was also dispensed into 3 empty centrifuge tubes to serve as blanks. The tubes containing the samples and $KMnO_4$ were capped and covered with aluminum foil before shaking for 1 min and 1, 3, 6, and 24 h on a reciprocal shaker (with tube lying on its side) or an end-over-end tumbler. The 1 min samples were filtered through a Whatman No. 1 filter paper until enough filtrate was collected for dilution and absorbance measurement. A corresponding blank sample was prepared which also passed through a Whatman no. 1 filter paper. The 1, 3, 6, and 24 h samples were centrifuged at 1030 x g for 5 min. Two-ml aliquots of KMnO4 from each sample and blank were transferred using a Pipetman™ into 50-ml volumetric flasks and diluted to volume. The absorbance of the samples and blanks was then measured at 565 nm. The concentration of KMnO4 from the samples and blanks was determined using the standard calibration curve. The amount of MnOC in the sample was computed as follows:

$$\text{MnOC (mg/g)} = \frac{(\text{mM Blank} - \text{mM Sample}) \times (50/2) \times 25 \times 9}{1000 \text{ (ml/l)} \times \text{wt of sample (g)}} \quad [3]$$

Where mM Blank and mM Sample are the concentrations (mmoles liter^{-1}) of $KMnO_4$ in the blank and sample, respectively, determined from the standard regression curve

50/2 = the dilution factor (ml/ml)

25 = the volume (ml) of KMnO4 added to the soil sample

9 = the amount of C oxidized for every mole of KMnO4 (g mole-1 or mg mmole-1)

(When $Mn^{+7}O^{4-}$ is reduced to $Mn^{+4}O_2$ and C is oxidized from the neutral state (0) to C^{+4}, 3 moles of C are oxidized for every 4 moles of Mn^{+7} reduced)

To check whether inorganic substances in the soil have some catalyzing effect on the oxidation of sugars by $KMnO_4$, 5 mg of C as glucose was mixed with air-dried soil samples containing 15 mg C, from Maahas, Bay and Pangil (Table 6.1) and was allowed to react with 33 mM $KMnO_4$ for 1 min. The MnOC in spiked (with glucose) and control (without glucose) soils were analyzed in triplicate.

Analysis of Permanganate-Oxidizable C in Organic materials

Three replicate samples of dried FYM, rice and wheat straw, sesbania and azolla, each containing 15 mg C were used to measure MnOC following the above procedure. Permanganate-oxidizable C was measured after 1 and 6 h.

Analysis of Permanganate-Oxidizable C in Soil Samples

Three replicate samples of 8 air-dried rice-field soils from Pila, Bugallon, Maahas, Maligaya, IRRI, Gapan, Luisiana, and Urdaneta (Table 6.1), each containing 15 mg C were used to measure MnOC following the above procedure. The amount of soil equivalent to 15 mg C was calculated from known total C contents of these 8 soils. Permanganate-oxidizable C was measured after 1, 3, 6 and 24 h. In some soils, the amount of dissolved organic C in the $KMnO_4$ extract was determined to obtain an estimate of the amount of C not completely oxidized to CO_2. One ml aliquot of the $KMnO_4$ extract after centrifugation was transferred into a 10-ml test tube, then 5 ml of acidified 0.2 M $FeSO_4$ was added to it to

reduce the excess $KMnO_4$. The amount of organic C in solution was then directly measured using 1020A combustion TOC analyzer from Oceanography International (OI) Corporation, College Station, Texas.

6.4.1.3 Biochemical and Physical Analysis of Soils

Particle size analysis was done by the pipette method and pH was measured in 1:1 soil:water suspension. Total C and N were determined by automated combustion using a Perkin-Elmer 2400 Elemental CHN analyzer from Perkin-Elmer Corporation, Norwalk, Connecticut (Jimenez and Ladha, 1993). Organic C was measured by adding 1 to 2 drops of 15% HCl to 60 mg soil sample in a silver capsule to convert carbonates to CO_2. The sample in the silver capsule was then dried in an oven at 80°C for about 2 h. The sample was sealed in the silver capsule and analyzed for C using the Perkin-Elmer 2400 CHN analyzer.

Water-soluble C was determined from 12 g of air-dried soil by shaking with 50 ml of deionized-distilled water for 1 h. The soil suspension was centrifuged for 30 min at 6953 x g and filtered through Whatman No. 42 filter paper. The total organic C in solution was measured using the 1020A combustion TOC Analyzer.

Microbial biomass C was measured by fumigation-extraction following the modified procedure of Witt et al. (1998) but using 10 g each of air-dried soil for the fumigated and unfumigated samples. The soils were incubated for one month under water-saturated conditions at 30°C prior to fumigation with chloroform.

All analyses were done in triplicate.

6.4.1.4 Statistical Analysis

Analysis of variance and linear regression analyses were done using SAS systems (SAS Institute, 1995). The F test for homogeneity of regression coefficients was used to determine if slopes of regression lines of MnOC vs. time (in natural log) obtained from different rice soils differed from one another (Gomez and Gomez, 1984).

6.4.2 Long-term effects of rice and wheat straw incorporation on soil parameters and soil quality in a rice-wheat system in Fukuoka, Japan

6.4.2.1 Soil and treatments

Soil samples were taken in October, 2003 after rice harvest from the long-term rice-wheat field experiment at the Kyushu National Agricultural Experiment Station, Chikugo, Fukuoka, Japan. The soil is of the Gray Lowland series with 29% clay, 56% silt and 16% sand. Details of the experiment are described in Tsuchiya et al. (2000). Soil samples for chemical analyses and laboratory incubation experiments were taken after rice harvest from the following treatments: 1) unfertilized control, 2) 70 kg N ha^{-1} as urea, 3) 10 Mg ha^{-1} rice straw incorporated, 4) 10 Mg ha^{-1} rice straw incorporated + 70 kg N ha^{-1} as urea, 5) 20 Mg ha^{-1} rice straw compost incorporated, 6) 20 Mg ha^{-1} rice straw compost incorporated + 70 kg N ha^{-1} as urea, 7) 6 Mg ha^{-1} wheat straw incorporated, and 8) 6 Mg ha^{-1} wheat straw incorporated + 70 kg N ha^{-1} as urea. For treatments 7 and 8, 10 Mg ha^{-1} of Italian ryegrass was incorporated from 1963 to 1984, then 10 Mg ha^{-1} of wheat straw from 1985 to 1993, and 6 Mg ha^{-1} of wheat straw from 1994 to 2003. The C and N contents of the organic materials incorporated into the soil are given in Table 6.2. The incorporation of the organic materials was done at puddling in mid June for rice. For composting, rice straw was cut into 5-10 cm pieces. Water was added and the straw was arranged into a pile. Mixing was done once every month. Rice straw harvested in October was incorporated as compost in June. Soil samples were air dried and ground to less than 2mm.

Table 6.2 Total C and N applied to the soil as organic amendments

Organic matter	Organic matter application rate Mg ha^{-1}	Total C content g kg^{-1}	Total N content g kg^{-1}	Total C applied per year kg ha^{-1}	Total N applied per year kg ha^{-1}	C/N mole ratio
Rice straw	10	348.0	6.46	3480	64.60	63
Rice straw compost	20	92.3	2.79	1846	55.80	39
Italian rye grass (22 yrs)[a]	8	288.0	13.70	2304	109.60	25
Wheat straw (9 yrs)[a]	10	416.0	3.74	4160	37.40	130
Wheat straw (9 yrs)[a]	6	416.0	3.74	2496	22.44	130

[a] In the Italian rye grass / wheat straw treatment, rye grass was incorporated during the first 22 y at the rate of 8 Mg ha^{-1} followed by wheat straw at a rate of 10 Mg ha^{-1} during the next 9 years, and wheat straw at a lower rate of 9 Mg ha^{-1} during the succeeding 9 years.

6.4.2.2 Grain yield measurement

Grain yield was determined from 60 hills per plot equivalent to 2.4 m^2. Grain weight was adjusted to 15% moisture.

6.4.2.3 Biochemical and physical analyses of air-dried soils

Particle size analysis, pH, total C and N, organic C, available P and K, and CEC was measured using procedures described in section 6.4.1.3

To obtain HWEC, 100 ml deionized-distilled water was added to 20g of dry soil and the mixture was boiled under reflux for 1 hr. The mixture was centrifuged at 2600*g* for 10 min. to obtain a clear centrifugate after which the water extract was analyzed directly for total and organic C using the1020A combustion TOC analyzer (Oceanography International Corporation, College Station, Texas).

Permanganate-oxidizable C was measured by shaking soil samples with 33 *m*M $KMnO_4$ for 6 h following the procedure in section 6.4.1.2

All soil samples were analyzed in triplicate.

6.4.2.4 Soil incubation experiments

Fifteen g portions of air-dried soil from each of the different treatments were incubated under aerobic and flooded conditions. For aerobic incubation, soils were placed in 4 cm diameter, 60 ml screw capped bottles, and water was added to 50% water-filled pore space (WFPS). For flooded conditions, soils were placed in 2.8 cm diameter, 60 ml tubes and then flooded with 15 ml of distilled water. Trapped air was removed by lightly tapping the tubes. Soils were incubated at 25 °C for 31 days.

To measure the flush of CO_2, a microcentrifuge tube containing 0.5 ml of 5N NaOH was placed inside each bottle with aerobic soil which was then tightly capped. For the flooded soils, 5N NaOH was placed inside a small glass tube closed at both ends but with a hole at the side. This was fitted into a hole at the center of a rubber stopper that was used to cover the 60-ml tube containing the flooded soil. After 3 days, the NaOH was transferred into a partially evacuated tube covered with serum cap, using a 1ml syringe, and acidified with excess HCl to release the trapped CO_2. The CO_2 evolved was then analyzed using a Hitachi gas chromatograph with a thermal conductivity detector. The column was packed with Porapak QS and the column temperature was kept at 50 °C. Helium was used as the carrier gas. A standard 10% CO_2 gas in helium was used for calibration.

After 30 days incubation, NaOH was again placed inside the soil containers to measure basal soil respiration for 24 hours. The same procedure as above was followed for measuring the CO_2 evolved.

After 31 days, MBC was measured in the aerobic and flooded soils by the fumigation-extraction method following the procedure used by Inubushi et al. (1991) except that the organic C in the fumigated and unfumigated K_2SO_4 extracts were measured using the the1020A combustion TOC analyzer.

Potential mineralizable N (NH_4^+ + NO_3^-) was determined 31 days after incubation from the K_2SO_4 soil extract after subtracting available N from the initial soil sample. NH_4^+-N was determined colorimetrically by the salicylate method (Kempers and Sweers 1986) and NO_3^- by the copperized cadmium reduction method (Page et al. 1982).

6.4.2.5 Statistical analyses

All statistical analyses were done using SigmaStat for Windows version 2.0 (Jandel Corporation). To compare the effects of organic residues and inorganic N treatments on rice yields and N uptake over several years, a three-way analysis of variance (organic matter x inorganic N x year) was performed together with mean comparison by the Tukey test.

To compare the effects of organic residues and inorganic N treatments on biochemical soil properties, a two-way analysis of variance (organic matter x inorganic N) was performed together with mean comparison by the Tukey test.

Simple linear regression analysis was done to determine yield trends and the relationships among the biochemical parameters used to assess soil quality.

6.4.3 Long-term effects of organic and inorganic fertilization on soil parameters and soil quality in rice-wheat systems in India and Nepal

6.4.3.1 Long-term Experiments

Two on-going rice-wheat long-term experiments in Ludhiana, India (Bhandari et al., 2002) and Bhairahawa, Nepal (Regmi et al., 2002) were used for this study. The Ludhiana experiment was initiated in June 1983 on a Typic Ustochrept (Tolewal loamy sand) at the experimental farm of the Punjab Agricultural University (PAU). The experiment included two crops per year, rice (July–

October) and wheat (November–April), with 11 treatments arranged in a randomized block design with three replications. The treatments comprised application of different combinations of inorganic and organic sources of nutrients to rice and wheat. In rice the full recommended levels of NPK (120:26:25) were supplemented with N through farmyard manure (FYM), wheat chopped straw (WS), and sesbania (*Sesbania cannabina* Linn. & Merrill) as green manure (GM) so that the 100% recommended N dose was available to the rice crop (Table 6.3). The wheat did not receive an organic amendment but received NPK (120:26:25). The Bhairahawa experiment was initiated in June 1988 on a Typic Haplaquept (calcareous) at the Agricultural Research Station, Bhairhawa, Nepal. The experiment also included two crops per year: rice (July-October) and wheat (November-March). There were six nutrient treatments comprising different combinations of N, P, FYM, and GM grown in situ arranged in a randomized complete block design with three replicates.

Of the 11 treatments in Ludhiana and 6 main treatments in Bhairahawa 5 in Ludhiana (LT 1 to LT5) and 6 in Bhairhawa (BT1 to BT3 with and without P) were selected for the present study (Table 6.3). The full experimental details of the two experiments have been published in earlier reports (Bhandari et al., 2002; Regmi et al., 2002).

6.4.3.2 Biochemical and physical analyses of air-dried soils

Air-dried soils collected after rice harvest in year 2003, 20 and 15 years after continuous applications of organic and inorganic fertilizers in Ludhiana and Bhairahawa, respectively, were analyzed for particle size, pH, CEC, available P and K, HWEC, MnOC, total C and N and dehydrogenase activity. The HWEC, MnOC, total C and N and dehydrogenase activity were also analyzed in archived soil samples collected after rice harvest during earlier years (starting from the 5^{th} y in Ludhiana and 3^{rd} y in Bhairahawa) to determine time trends.

Particle size, pH, total C and N, Organic C, available P and K, and CEC were measured following the procedures described in section 6.4.1.3 and HWEC were measured following the procedures described in section 6.4.2.3.

Permanganate-oxidizable C was determined by shaking soil samples with 33 *m*M KMnO4 for 6 h. following the procedure described in section 6.4.1.2, and

Table 6.3 Selected treatments from the Ludhiana and Bhairahawa LTE

Treatment		Rice						Wheat						Rice+Wheat		
		N		P		K		N		P		K		N	P	K
		I[††]	O[§]	I	O	I	O	I	O	I	O	I	O	I + O		
Code	Details[†]	kg ha^{-1}														
Ludhiana																
LT1	Unfertilized control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LT2	100% NPK in R&W[¶]	120	0	26	0	25	0	120	0	26	0	25	0	240	52	50
LT3	50% NPK + 6 Mg ha^{-1} FYM in R, 100% NPK in W	60	60	13	18	13	30	120	0	26	0	25	0	240	57	68
LT4	75% N + 50% PK + 6 Mg ha^{-1} WCS in R, 100% NPK in W	90	30	13	6	13	18	120	0	26	0	25	0	240	45	56
LT5	50% NPK + 50% N with GM in R, 100% NPK in W	60	60	13	6	13	54	120	0	26	0	25	0	240	45	92
Bhairahawa																
BT1-P	Inorganic N in R&W	100	0	0	0	0	0	100	0	0	0	0	0	200	0	0
BT1+P	Inorganic N and P in R&W	100	0	26	0	0	0	100	0	26	0	0	0	200	52	0
BT2-P	Inorganic N in R&W and GM in R	100	33	0	3.3	0	30	100	0	0	0	0	0	239	3.3	30
BT2+P	Inorganic N and P in R&W and GM in R	100	60	26	6	0	54	100	0	26	0	0	0	260	58	54
BT3-P	Inorganic N in R&W and FYM in W	100	0	0	0	0	0	100	80	0	20	0	40	280	20	40
BT3+P	Inorganic N and P in R&W and FYM in W	100	0	26	0	0	0	100	80	26	20	0	40	280	72	40

[†] R = rice; W = wheat; FYM = farmyard manure; WCS = wheat cropped straw; GM = green manure (*Sesbania aculeata*)

[††] I = inorganic nutrient

[§] O = organic nutrient

[¶] 100% recommended N, P, and K for rice and wheat were 120, 26.2, and 25 kg ha^{-1}, respectively. Beginning in 1992, application of P was reduced to 13.1 kg ha^{-1} in rice.

dehydrogenase activity was determined using 6g of air dried soil following the method of Tabatabai (1982). All soil samples were analyzed in triplicate.

6.4.3.3 Soil incubation experiments

The same procedures used for the Japan LTE as described in section 6.4.2.4 were applied.

6.4.3.4 Statistical analyses

All statistical analyses were done using SigmaStat for Windows version 2.0 (Jandel Corporation).

6.5 Results

6.5.1 Assessing the Reliability of Permanganate-oxidizable C as an Index of Soil Labile C

6.5.1.1 Oxidative Reaction of Simple Organic Compounds with $KMnO_4$

Reactivity with 33 *mM* $KMnO_4$ varied among the organic compounds tested based on the change in $KMnO_4$ concentration. Generally, the reaction followed a logarithmic trend, and started to plateau after 4-5 h (Fig. 6.2). For the sugars tested, only 2-41% C was oxidized to CO_2 after 1 h in the order arabinose > xylose > fructose > mannose > glucose > maltose > sucrose. The disaccharides maltose and sucrose are considered to be non-reducing sugars because they lack a free aldehyde or keto group. However, they may be oxidized after undergoing hydrolysis to glucose and fructose (for sucrose). Thus, maltose and sucrose were found to be the least reactive with neutral $KMnO_4$ with only 2-3% C oxidized to CO_2 in 1 h and 23% in 24 h (Fig. 6.2a). Increasing the concentration of $KMnO_4$ to 333 *mM*, at most, doubled the amount of C oxidized in monosaccharides (data not shown). For the amino acids tested, only 2-25% C was oxidized by $KMnO_4$ after 1 h with threonine being the most reactive and valine the least (Fig. 6.2b). Among the organic acids, gluconic acid was oxidized by $KMnO_4$ to the greatest extent (45% C in 1 h and 100% in 24 h), whereas benzoic acid was unreactive. For oxalic acid, 20% C was oxidized in 1 h but this did not increase further up to 24 h (Fig. 6.2c). Cellulose was unreactive with 33 and 333 *mM* $KMnO_4$ (data not shown) as it has only one free aldehyde or keto group at the reducing end. In addition, cellulose > 6 glucose units are not soluble. Potassium permanganate was used to separate lignin and cellulose in acid detergent fiber (Van Soest and Wine,

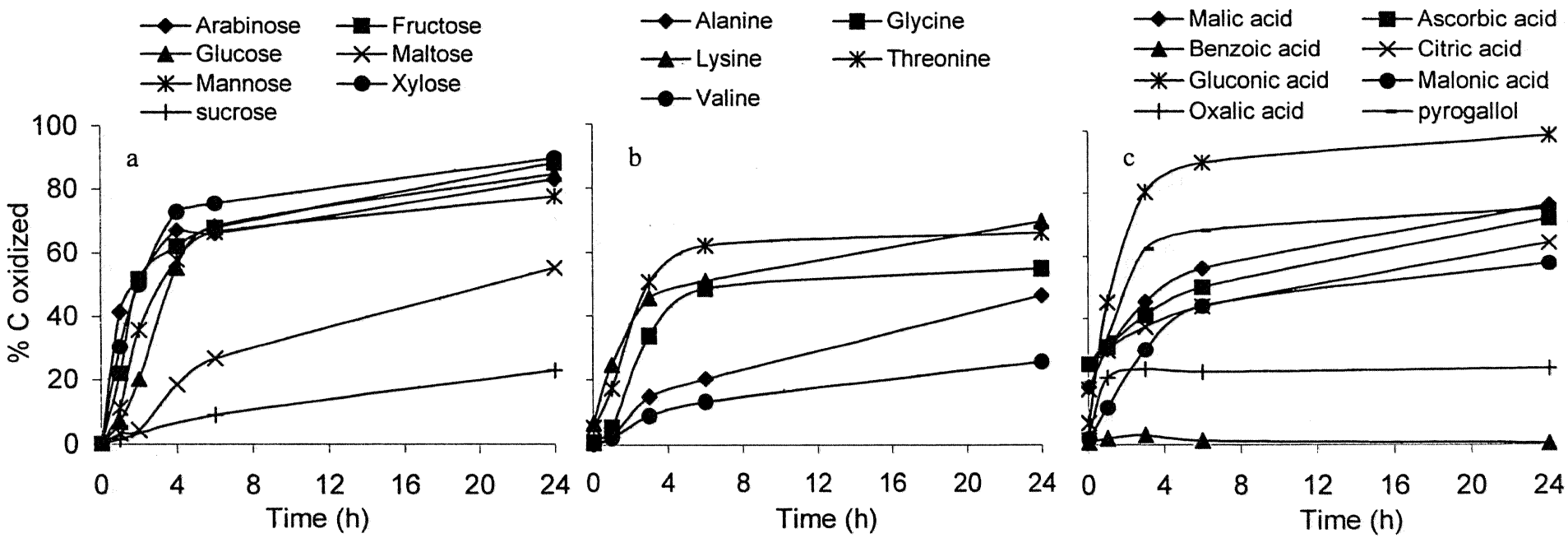


Fig. 6.2 Kinetics of organic C oxidation by 33 *mM* $KMnO_4$ (0-24 h) at room temperature in various organic compounds: a) sugars, b) amino acids, c) organic acids.

1968) because of the difference in reactivity of lignin and cellulose to $KMnO_4$ oxidation.

Benzene carboxylic acids are considered to be permanganate-resistant molecules, whereas alkanoic acids and phenolic acids are progressively broken down into short dicarboxylic molecules, for example, oxalic acid (Almendros et al., 1989). Among the organic compounds tested, pyrogallol and ascorbic acid showed the quickest reaction with $KMnO_4$ (25 % C oxidized in 1 min) (Table 6.4). Both contain multiple glycol groups that are quickly oxidized by $KMnO_4$. In contrast, no detectable amount of C was oxidized by $KMnO_4$ after 1 min among the sugars. For the amino acids and the other organic acids, 4-7% and 0.6-18% C, respectively, were oxidized in 1 min by 33 *mM* $KMnO_4$ (Table 6.4).

Table 6.4 One-minute oxidation of various organic compounds with 33 *mM* $KMnO_4$.

Organic Compound	% C oxidized to CO_2 based on the change in $KMnO_4$ concentration
Alanine	4.5 ± 5.4†
Glycine	4.3 ± 3.8
Lysine	6.5 ± 3.6
Threonine	6.2 ± 3.4
Valine	4.2 ± 3.5
Malic Acid	17.9 ± 2.6
Ascorbic Acid	25.5 ± 0.5
Benzoic Acid	0.6 ± 1.3
Citric Acid	17.2 ± 0.7
Gluconic Acid	6.8 ± 0.5
Malonic Acid	2.0 ± 0.9
Oxalic Acid	3.6 ± 1.3
Pyrogallol	25.4 ± 0.3

† ± standard deviation

One minute shaking with $KMnO_4$ was not long enough to oxidize glucose that was mixed with the soil judging from the observation that there was no difference in the amount of C oxidized from soils spiked with glucose and from those that were not spiked (Table 6.5). About 1 mg C in Maahas and Pangil and 3 mg C per g dry soil in Bay were oxidized by $KMnO_4$. The estimated amount of soil C oxidized by $KMnO_4$ in 1 min exceeded the water-soluble C content in the three soils tested (Table 6.5), suggesting that $KMnO_4$ more rapidly oxidized less available organic compounds.

Table 6.5 One-minute oxidation of soil organic C with 33 *mM* $KMnO_4$ in three different rice soils.

Soil	Oxidized C based on $KMnO_4$ consumed‡		Water-soluble C	C in $KMnO_4$ extract (B)	C oxidized to CO_2 (A - B)
	Spiked	Unspiked(A)			
	g kg^{-1}				
Maahas	1.2 ± 0.2†	1.1 ± 0.1	0.016 ± 0.007	0.9 ± 0.3	0.2 ± 0.3
Bay	3.1 ± 0.2	3.1 ± 0.1	0.091 ± 0.042	2.1 ± 0.3	1.0 ± 0.3
Pangil	0.9 ± 0.1	1.1 ± 0.1	0.066 ± 0.007	0.4 ± 0.2	0.7 ± 0.2

† ± standard deviation

‡ Spiked, 10 mg C (as glucose) g^{-1} dry soil was added to each of the soil samples; unspiked, glucose was not added.

Since air-dried soils were used, it is unlikely that there were reduced ions in these soils that could have reacted with $KMnO_4$.

The above results show that oxidation of sugars and amino acids by neutral $KMnO_4$ does not proceed very rapidly and that the reactivity of water-soluble organic compounds with $KMnO_4$ varies depending on their structure and functional groups. One h of oxidation with 33 or 333 *mM* $KMnO_4$ cannot discriminate labile from non-labile C because of the inability of neutral $KMnO_4$ to oxidize some simple sugars on the one hand and its ability to oxidize less readily metabolized organic compounds on the other hand. Data obtained suggested that neutral $KMnO_4$ strongly oxidized a bigger pool of less readily metabolized organic compounds. Thus, MnOC is not a good measure of the active, labile, or available C fraction.

Maximov et al. (1977) showed the predominant degradation by $KMnO_4$ of compounds containing glycol groups in humic acids. In agreement, we found that ascorbic acid and pyrogallol, which contained multiple glycol groups, were more rapidly oxidized by $KMnO_4$ (in 1 min) than the other organic acids and sugars. Conteh et al. (1999) have also shown that $KMnO_4$-oxidizable C contains polysaccharide and humic C and it is an order of magnitude greater than the K_2SO_4-extractable or microbial biomass C fractions.

Unprotected glycol groupings and double bonds are usually attacked by alkaline $KMnO_4$, often resulting in C-C bond cleavage (Green, 1980). Under controlled conditions, $KMnO_4$ effects *cis*-hydroxylation of double bonds. Primary and secondary alcohols are also rapidly oxidized by alkaline $KMnO_4$; the reaction is slower in neutral and mildly acidic solutions. The rapid oxidation in alkaline solution has been attributed to the formation of a nucleophilic alkoxide anion. D-glucose may be

oxidized completely to CO_2 and water by hot, weakly alkaline solutions of $KMnO_4$; as the alkalinity increases above 0.03 *N*, oxalic acid is produced, and, in 1.8 *N* KOH, yields of 42% oxalic acid are obtained (Green, 1980).

6.5.1.2 Oxidation of Complex Organic Compounds by $KMnO_4$

We tested the susceptibility of various organic materials (crop residue [rice and wheat straw], farmyard manure [FYM], and green manure [azolla and sesbania]) to oxidation by $KMnO_4$ (Table 6.6). Based on the amount of $KMnO_4$ consumed, azolla was the most susceptible to oxidation, having the highest MnOC (65 and 99 mg g^{-1} in 1 and 6 h, respectively) and MnOC/TC (14.5% in 1 h and 22.3% in 6 h), whereas sesbania was the least susceptible, with an MnOC of 31.1 mg g^{-1} in 1 h and 65.6 mg g^{-1} in 6 h. The MnOC of the organic materials did not correlate with their total C content but correlated ($P < 0.05$) with their lignin content (Fig. 6.3). The C/N and lignin/N ratios have been shown to be negatively correlated with the mineralization rates of organic manures (Becker et al., 1994). Thus, rice and wheat straw, having high C/N (55 and 125, respectively) and lignin/N (12 and 32, respectively) ratios, have lower mineralization rates than sesbania, azolla, and FYM with low C/N (27, 13, and 20, respectively) and lignin/N (3, 5, and 5, respectively) ratios. On the contrary, rice and wheat straw had higher $KMnO_4$ oxidation rates (36 and 48 mg MnOC g^{-1} h^{-1}, respectively) than sesbania (31 mg MnOC g^{-1} h^{-1}). Permanganate-oxidizable C was not correlated with the lignin/N nor with the C/N ratios of the organic manures tested, suggesting that MnOC is not predictive of mineralizable C but may indicate the lignified nature of the organic material.

Complex organic compounds in the soil are not completely oxidized to CO_2 by $KMnO_4$. Organic C was detected in the soil $KMnO_4$ extracts in amounts greater than the water-soluble C, suggesting that intermediate products of oxidation were formed (Table 6.5). Thus, for complex organic materials, it may be difficult to estimate the amount of C oxidized to CO_2 from the amount of $KMnO_4$ consumed since $KMnO_4$ results in the formation of intermediate C compounds other than CO_2. Loginow et al. (1987) have shown that the estimated amount of MnOC from various organic materials was generally higher when measured on the basis of $KMnO_4$ consumption than the actual amount of CO_2 evolved. They also found that lignin used more oxidizer than cellulose although the amounts of CO_2 evolved from these organic materials were similar, which suggests a portion of $KMnO_4$ consumed by lignin

Table 6.6 Total C and permanganate-oxidizable C (MnOC) in some organic materials.

Organic Materials	Total C	Total N	C/N	Lignin	Lignin/N	1 h		6 h	
						MnOC	MnOC/TC	MnOC	MnOC/TC
	g kg^{-1}		mole ratio	g kg^{-1}	g g^{-1}	g kg^{-1}	%	g kg^{-1}	%
Farmyard Manure	325	19.3	19.6	101[†]	5.2	45.4 ± 1.0[§]	15.4 ± 1.8	76.2 ± 1.7	25.7 ± 1.9
Rice Straw	373	8.0	54.6	93[‡]	11.6	35.5 ± 0.6	8.9 ± 0.5	66.2 ± 0.2	16.5 ± 1.2
Wheat Straw	458	4.4	121.0	140[†]	31.7	47.4 ± 1.8	10.5 ± 1.1	88.0 ± 1.1	19.5 ± 0.0
Azolla	444	41.5	12.5	185[‡]	4.5	64.7 ± 0.9	14.5 ± 0.2	99.4 ± 0.3	22.3 ± 0.2
Sesbania Rostrata	452	19.9	26.5	55[‡]	2.8	31.1 ± 1.8	6.9 ± 0.4	65.6 ± 0.7	14.5 ± 0.2

[†] Data were taken from Cornell Waste Management Institute, 2000

[‡] Data were taken from Becker et al., 1994. Lignin was determined by the acid detergent method (Van Soest, 1968).

[§] ± standard deviation

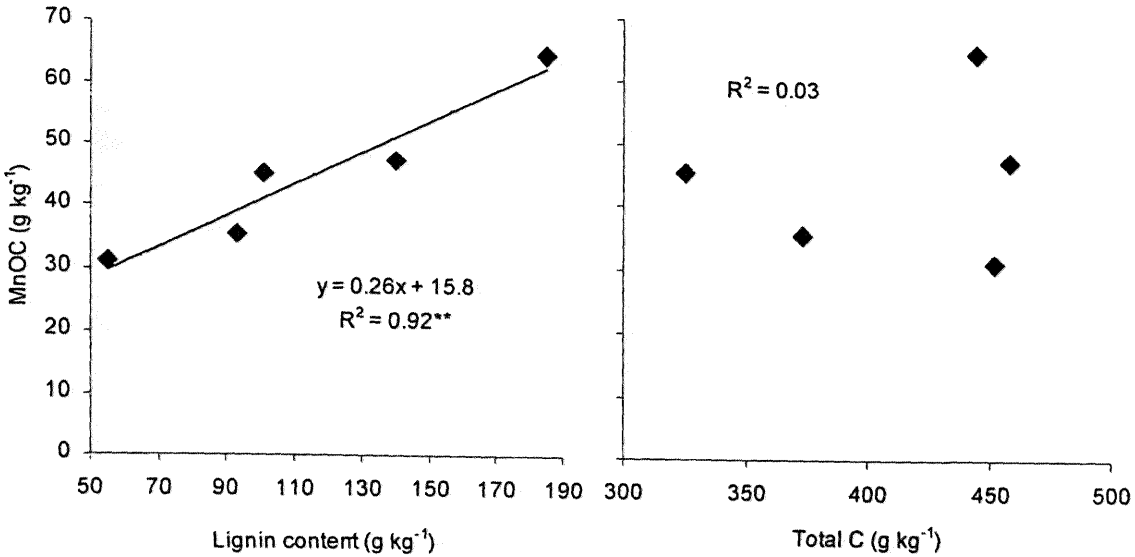


Fig. 6.3 Relationship of MnOC in FYM, rice and wheat straw, azolla and sesbania with their a) lignin and b) total C contents.

produces lower molecular weight organic compounds. In contrast, cellulose may be broken down into glucose units, which can be completely oxidized to CO_2. The three monomers that make up almost all lignin found in nature are *p*-coumaryl, coniferyl, and sinapyl alchohol (Barker and Owen, 1999). Lignin contains glycol groups and double bonds that are rapidly oxidized by $KMnO_4$. Thus, lignin is more susceptible to $KMnO_4$ oxidation than cellulose although cellulose is more susceptible to microbial decomposition (Bohn et al., 1985).

6.5.1.2 Soil MnOC as Affected by Total Soil C and Soil Texture

The MnOC measured after 1, 3, 6, and 24 h was compared in 8 different rice-field soils. The amount of C oxidized by 33 *mM* $KMnO_4$ increased with time following a logarithmic trend (Fig. 6.4). The F test for homogeneity of regression coefficients gave a highly significant ($P<0.01$) F value indicating that the slopes of the regression lines of MnOC vs. time in natural log varied among the different rice soils. The 95% confidence intervals of the slopes confirm significant differences in $KMnO_4$ oxidation rates of these soils. The oxidation rate (slope) was highly correlated with the total organic C content ($R^2 = 0.94$, $P<0.01$) (Fig. 6.5). The total cumulative MnOC as a fraction of total organic C (MnOC/TC) ranged from 8% to 14% after 1 h, from 11% to 19% after 3 h, from 15% to 23% after 6 h, and from 23% to 33% after 24 h.

Soil MnOC of 10 soils from the Philippines together with soil MnOC from plots with different organic matter treatments in two rice-wheat long-term experimental sites in India, were regressed with their total C contents and were found to be highly correlated ($R^2=0.96$, $P<0.01$)(Fig. 6.6). Regression analyses showed that the standard error of the estimate was higher across different soil types than within the same soil with different organic fertilizer treatments.

The effect of soil texture on soil MnOC was examined by analyzing the relationships of clay/OC, silt/OC and clay+silt/OC ratios with soil MnOC in 12 different rice-field soils from the Philippines and India. The scatter plots in Fig. 6.7 show two points with a large deviation from the regression line. These two soils contained extreme OC contents: Bay with a very high OC content of 49.6 g kg^{-1}) and Ludhiana with a very low OC content of 4.35 g kg^{-1}. For soils with very low or very high soil OC, soil texture may not have any significant effect on MnOC. The clay/OC ratio had no significant correlation with soil MnOC even if the soils with extreme OC contents were excluded from regression analysis. However, silt/OC and silt+clay/OC

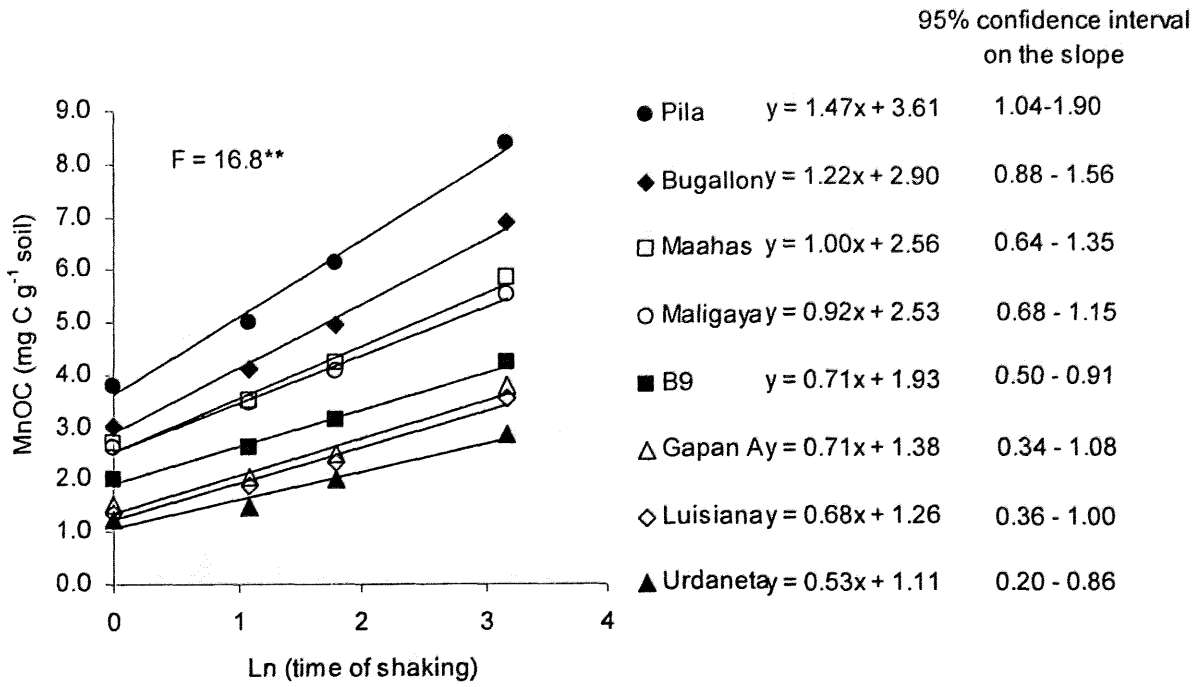


Fig. 6.4 Kinetics of soil organic matter oxidation by 33 mM $KMnO_4$ at room temperature in 8 rice-field soils (** slopes are significantly different at the 1% level by the F test for homogeneity of regression coefficients).

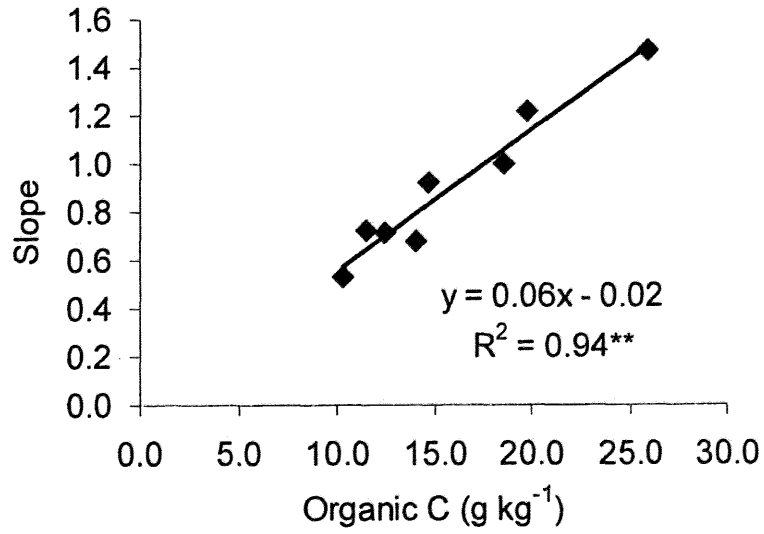


Fig 6.5 Relationship between total C content and the oxidation rate of soil organic C by $KMnO_4$ in 8 rice field soils.

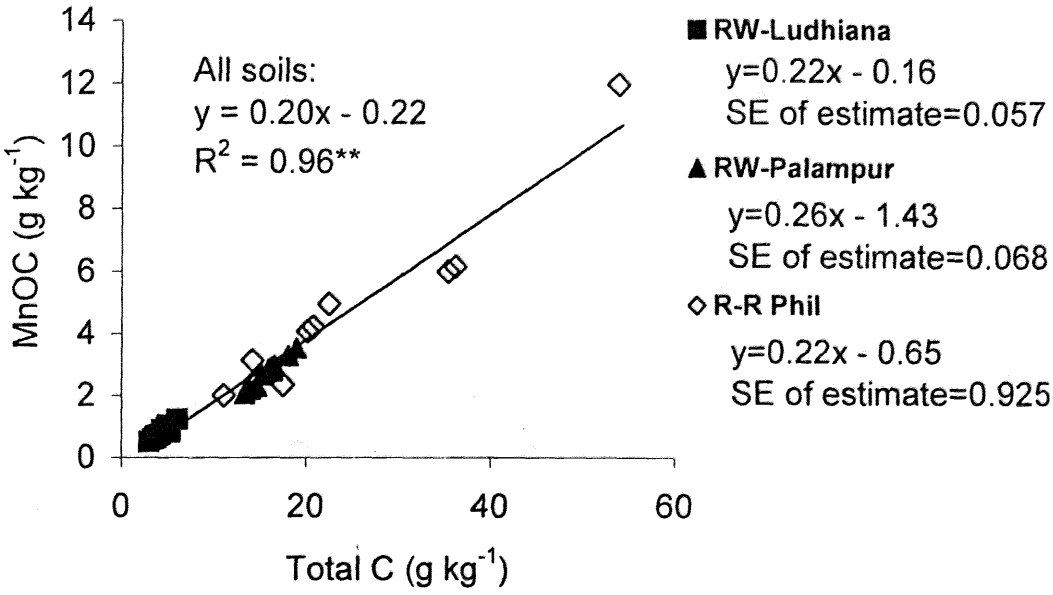


Fig. 6.6 Relationship of soil MnOC and total C in 10 different rice-field soils from the Philippines and rice-wheat field soils with different organic fertilizer treatments in 2 sites in India.

had a significant ($P<0.01$) inverse relationship with soil MnOC if the two soils were excluded. Silt+clay/OC accounts for 82% of the variability in soil MnOC in 10 soils (Fig. 6.7).

The above results show that although soil MnOC is predominantly influenced by total soil C, it is also affected by soil texture as shown by its high correlation with the (clay + silt) / OC ratio. Clay and silt particles may render some degree of physical protection for oxidizable C groups in lignin.

6.5.1.3 Soil MnOC as Affected by Organic Fertilizer Treatments

Soil MnOC and total C were measured in a long-term rice experiment at IRRI after 27 and 29 rice crops. Azolla and sesbania fertilization increased soil MnOC over the control and urea treatments mainly due to the added MnOC from azolla and sesbania. The total MnOC added to the soil was 5.2 g kg^{-1} soil from azolla and 5.9 g kg^{-1} soil from sesbania (Table 6.7). However, the measured soil MnOC from the azolla and sesbania treatments was lower than that estimated from the added MnOC of the GM. About 48 and 43 % of MnOC from azolla, and 48 and 51 % of MnOC from sesbania in 1997 and 1999 respectively, was lost from the upper 0-20 cm soil

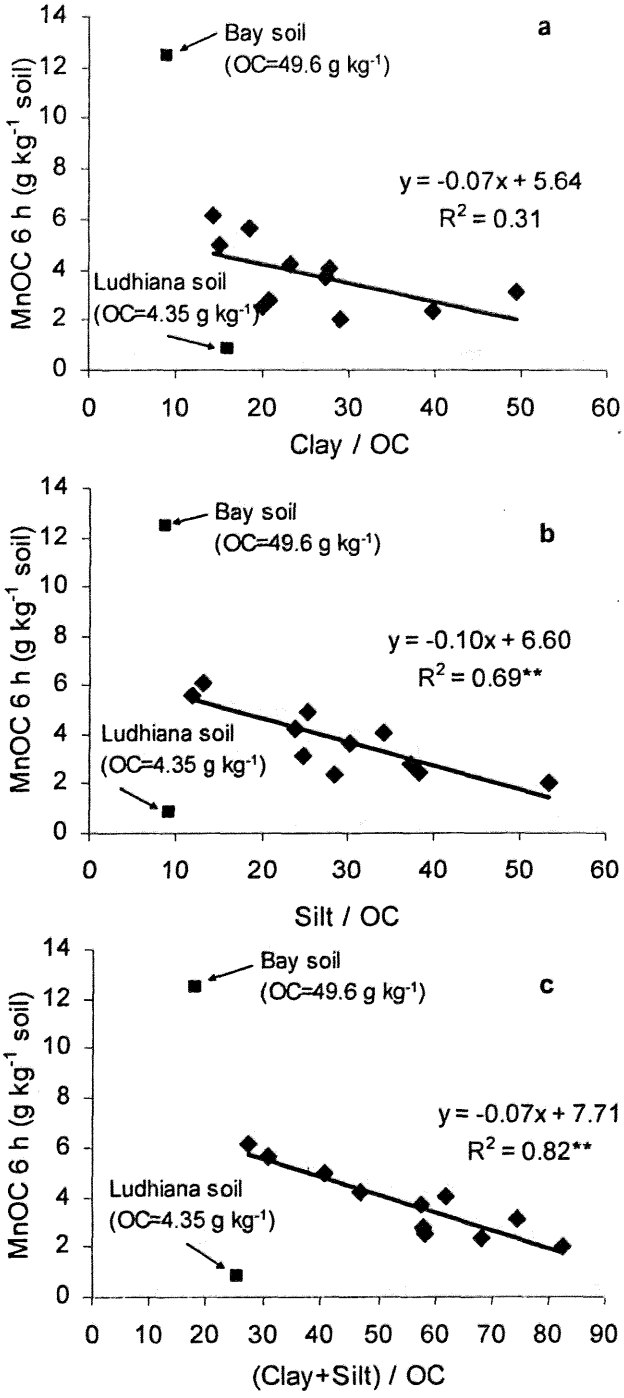


Fig. 6.7 Scatter plots and linear regression of soil MnOC with a) clay/OC, b) silt/OC and c) clay+silt/OC.

Table 6.7 Soil MnOC as affected by organic and inorganic fertilization after 27 and 29 crops in a long-term double rice cropping at IRRI.

Treatment	Total Soil C[1] (g kg^{-1})	Measured Soil MnOC[1] (g kg^{-1}) (A)	Amount of GM added (g GM kg^{-1} soil)	MnOC conc. of added GM (g POC kg^{-1} GM)	Total MnOC from added GM (g kg^{-1} soil) (B)	MnOC of control + MnOC from added GM (g kg^{-1} soil) (C)
1997 (27 crops)						
Control	18.8b	3.9c	-	-	-	-
Urea	17.6b	3.9c	-	-	-	-
Azolla	20.9a	4.7b	52	99.4	5.2	9.1
Sesbania	21.7a	5.1a	90	65.6	5.9	9.8
1999(29 crops)						
Control	18.9b	4.3b	-	-	-	-
Urea	19.1b	4.4b	-	-	-	-
Azolla	24.2a	5.9a	61	99.4	6.1	10.4
Sesbania	23.8a	5.6a	109	65.6	7.2	11.5

[1] Means followed by a common letter are not significantly different from each other at the 5% level by Duncan's multiple range test.

depth (computed from data in Table 6.7: (C-A) x 100 / C). Some of the MnOC may have moved down to the lower soil depth as the azolla and sesbania treatments also had higher MnOC than the control and urea treatments in the 20-50 cm soil depths (2.6 mg g^{-1} in the control and urea treatments and 3.1 mg g^{-1} in the azolla and sesbania treatments). The unaccounted MnOC may have been lost or was stabilized in the soil clay particles. In terms of total amount of GM and total C added (azolla has a total C content of 42.5% while sesbania has a total C content of 44.2%), more was added from sesbania as compared to azolla but the soil C from the two treatments were similar showing greater C loss from sesbania (Table 6.7).

In a rice-wheat long-term experiment in Ludhiana, India, the effect of organic fertilizer treatments on total soil C and MnOC over 16 years was determined. Analyses of variance over years showed significant differences among years and treatments in terms of total C, MnOC and the MnOC/TC measured after 6 h, without any treatment x year interaction (Table 6.8). All the organic fertilizer treatments (FYM, wheat straw, and GM) had significantly higher total soil C and MnOC than the unfertilized treatment. However, in terms of MnOC/TC, only the FYM treatment was significantly different from the control and the 100% NPK treatment. The increase in total soil C and MnOC in the fertilized over the control plots could be due to C

Table 6.8 Effect of agronomic treatment on total C and MnOC in a long-term experiment In Ludhiana, India.

Year/Treatment	Total C ($g\ kg^{-1}$)	6 h MnOC ($g\ kg^{-1}$)	6 h MnOC/Total C (%)
Year	Average of 5 treatments		
1983	3.6	0.6	17.1
1988	4.5	0.9	18.7
1991	4.9	0.9	18.9
1993	5.0	0.9	18.5
1995	4.9	0.9	19.1
1998	5.2	1.0	18.3
1999	5.3	1.0	18.9
SE	0.20	0.04	0.35
Treatment	Average of 7 years		
Control	4.1	0.7	17.9
100% NPK	4.7	0.8	17.9
50% NPK + 6Mg ha^{-1} FYM	5.3	1.0	19.2
75% N + 50% PK + 6Mg ha^{-1} wheat straw	4.8	0.9	18.9
50% NPK + 50% N from GM (Jantar)	4.9	0.9	18.6
SE	0.16	0.04	0.31
ANOVA over 6 years: Source of variation	F Value		
Year	5.9**	9.8**	3.7**
Treatment	6.3**	8.5**	3.6*
Year x Treatment	1.4ns	1.8*	1.4ns

sequestration from added organic fertilizers and increase in root residues in fertilized plots. Increase in MnOC/TC in the FYM treatment could be due to the higher MnOC/TC of pure FYM (25.7%) (Table 6.6) as compared with the control soil (17.9%). One-hour oxidation did not show significant differences among treatments and years in terms of MnOC/TC (data not shown).

These results show that soil MnOC is affected by the MnOC of the organic material added to the soil. In these experiments, the effect of organic fertilizers was evident not only in the change in soil MnOC but in the change in total soil C as well. Thus, MnOC cannot really be claimed as a more sensitive indicator of the changes in the labile C pool than total C.

6.5.1.4 Relationships of Soil MnOC with WSC, MBC

The relationships of soil MnOC with total C, WSC, and MBC were determined using eight rice-field soils from the Philippines (Fig. 6.8). Permanganate-oxidizable C

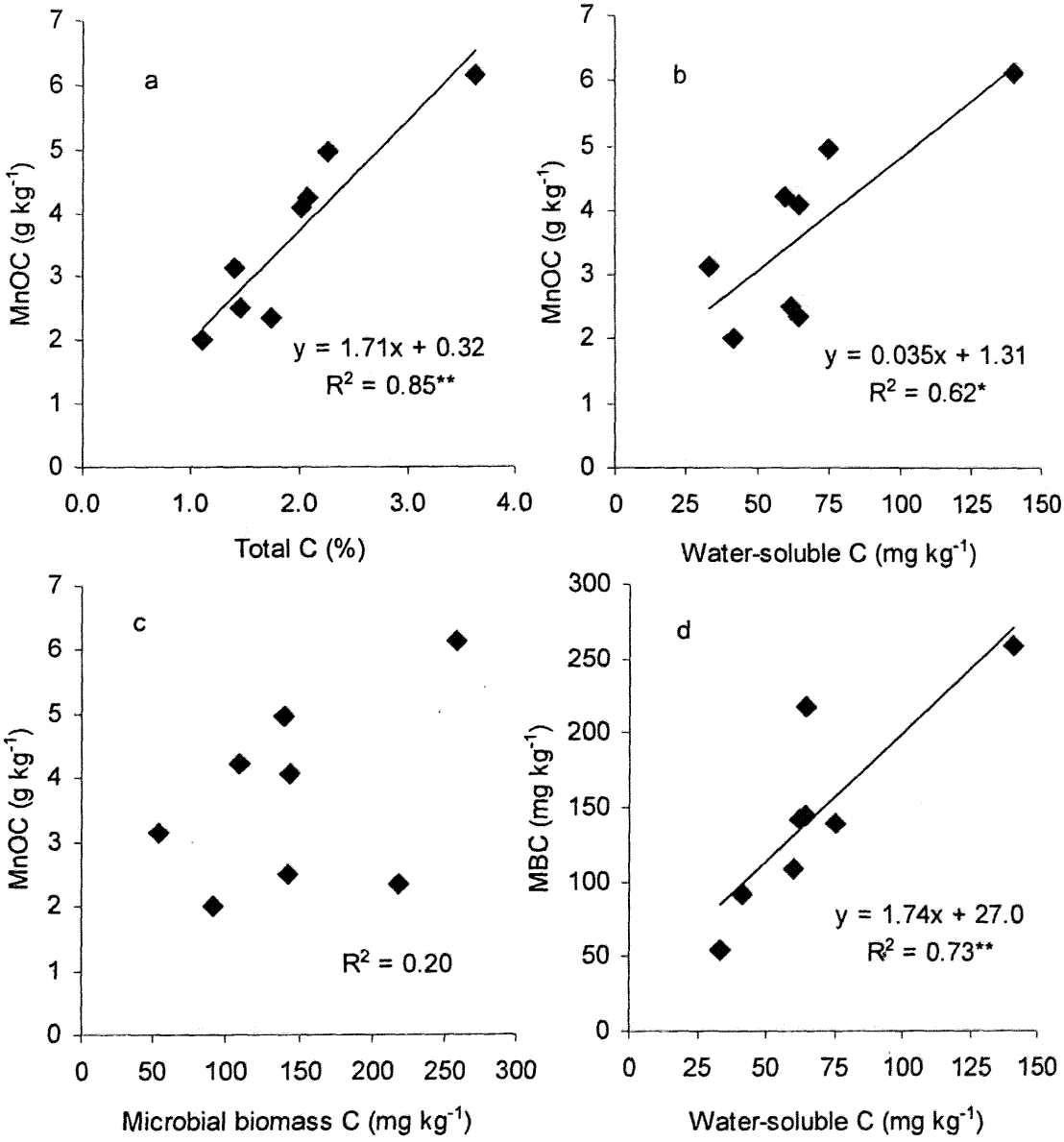


Fig. 6.8 Relationship of soil MnOC with a) total C, b) WSC, and c) MBC, and d) MBC with WSC in 8 rice-field soils.

was significantly correlated with both total C and WSC. However, MnOC had a better correlation with total C ($P < 0.01$) than with WSC ($P < 0.05$). The significant correlation between MnOC and WSC is dictated by only one point (Pila soil which has the highest MnOC and WSC). The correlation becomes insignificant at $P<0.05$ if this point is removed. Moreover, MnOC was not correlated with MBC, whereas WSC

was significantly correlated with MBC. The high correlation between MBC and WSC is also dictated by Pila soil. Removing this point lowers the correlation coefficient but it will still be significant at the 5% level.

The lack of correlation of MnOC with WSC and MBC and its high correlation with total C further suggest that MnOC does not measure labile C but may represent a more stable fraction of total C. Permanganate-oxidizable C appears to be a better indicator of lignin content than labile C and thus it may also be used to monitor soil quality and sustainability by showing changes in the stored organic matter or the slow C pool resulting from various agronomic practices. Permanganate-oxidizable C may also indicate the extent of physical protection of clay particles for oxidizable C in lignin.

6.5.2. Long-term effects of rice and wheat straw incorporation on soil parameters and soil quality in a rice-wheat system in Fukuoka, Japan

6.5.2.1 soil pH, CEC, available P and K

Long-term application of rice and wheat residues significantly reduced the soil pH from 5.7 in the unfertilized control to the lowest value of 5.2 in the wheat straw treatment combined with inorganic N. Treatments with inorganic N had lower pH than those without. On the other hand, the organic residue treatments increased CEC but inorganic N had no significant effect on CEC. The rice straw compost treatment had the highest CEC among the organic residue treatments (Table 6.9).

Residue incorporation significantly increased both available soil P and K. The highest available soil P content was observed in the wheat straw treatment. Olsen P was significantly higher in the wheat straw than in the rice straw and rice straw compost treatments. The rice and wheat straw treatments had similar soil available K contents that were higher than the control. However, soil available K was lower in the composted rice straw treatment than that in the rice straw treatment indicating loss of K during composting (Table 6.9).

6.5.2.2 Total C and N

The total amount of C applied to the soil annually was highest from rice straw (3.5 Mg ha-1) and least from rice straw compost (1.8 Mg ha-1)(Table 6.2). However, the long-term application of rice and rye grass / wheat straw increased the total soil C

Table 6.9 Effect of long term organic matter treatment on pH Olsen P and available K (Fukuoka LTE, 2003).

Treatment	pH						Olsen P mg $100g^{-1}$						Ex-K mg $100g^{-1}$						CEC meq $100g^{-1}$					
	-N		+N		mean		-N		+N		mean		-N		+N		mean		-N	+N	mean			
Control	5.7	a	5.4	a*	5.6		7.9	c	7.9	c	7.9		16.3	b	10.5	d*	13.4		17.0	17.4	17.2	c		
Rice Straw	5.5	bc	5.3	b*	5.4		8.9	b	9.1	b*	9.1		20.9	a	19.1	a*	19.9		18.1	18.3	18.2	b		
Rice Straw Compost	5.4	c	5.3	b*	5.4		8.6	b	8.9	b*	8.9		16.3	b	11.7	c*	13.9		19.8	19.8	19.8	a		
Rye grass/Wheat Straw	5.5	b	5.2	b*	5.4		10.7	a	10.8	a	10.8		21.4	a	16.0	b*	18.8		18.0	18.2	18.1	b		
Mean	5.5		5.3	*			90.5		92.8	*			18.7		14.4	*			18.2	18.4				

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

* Effect of N is significant at the 5% level by Tukey test.

Table 6.10 Effect of long term organic matter treatment on total soil C and N (Fukuoka LTE, 2003)

Treatment	Total C (mg g^{-1} dry soil)						Org C (mg g^{-1} dry soil)						Total N (mg g^{-1} dry soil)						C/N	
	-N		+N		mean		-N		+N		mean		-N		+N		mean		-N	+N
Control	19.90	c	21.43	c*	20.70		18.10		19.07		18.58	c	1.82	c	1.93	c*	1.88		12.8	13.0
Rice Straw	24.90	b	25.70	b*	25.30		22.90		23.13		23.02	b	2.25	b	2.34	b*	2.30		12.9	12.8
Rice Straw Compost	28.50	a	30.10	a*	29.30		25.93		27.07		26.50	a	2.59	a	2.76	a*	2.68		12.8	12.7
Rye grass/Wheat Straw	25.10	b	25.80	b*	25.40		23.27		23.70		23.48	b	2.24	b	2.36	b*	2.30		13.1	12.8
Mean	24.60		25.80	*			22.55		23.24	*			2.23		2.35	*			12.9	12.8

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

* Effect of N is significant at the 5% level by Tukey test.

content by only 25-26% in the –N treatments and 20% in the +N treatments (Table 6.10) relative to the control, while the application of rice straw compost resulted in a bigger increase in total soil C of 43% in the –N and 40% in the +N treatments. These results show that the quantity of C returned to the soil cannot be a good indicator of C retention. Similarly, significant increases in total soil N were observed with the application of rice and rye grass / wheat straw (21-24%) in the –N and +N treatments and a much higher increase with the application of rice straw compost (42-43%).

The estimated percentage of total C retained in the soil from rice straw compost (23%) was highest as compared with that from rice straw (7%) and rye grass / wheat straw (9%). Similarly the percentage of total N retained in the soil was also highest from composted rice straw (72%) (Table 6.11).

6.5.2.3 Microbial biomass C, soil respiration rate and metabolic quotient

The effects of different organic fertilizer treatments on microbial biomass C was compared in air-dried soils after pre-incubation for 1 month under aerobic (50% WFPS) and flooded conditions. Analysis of variance showed a significant effect of residue treatment on MBC, but no significant effect of inorganic N and no significant interaction between organic and inorganic treatments. The MBC of soils in all the residue treatments were significantly higher than that of the control under aerobic condition. On the other hand, increased MBC over the control was observed only with composted rice straw under flooded condition. Further, MBC expressed as a percentage of total C did not vary among the treatments under aerobic (4%) and flooded (3%) conditions (Table 6.12) showing that MBC increased proportionately with total C.

Soil respiration rates and metabolic quotients (qCO_2 = respiration rate per unit biomass) were measured in the different organic residue treatments and the control in order to assess microbial activity and the efficiency by which available C is utilized by the soil micro biota. Basal soil respiration rate was significantly higher in the rice straw compost treatment as compared with the control under aerobic condition but no significant differences among treatments were observed under flooded condition (Table 6.13). However, basal soil respiration rate was reduced by the addition of inorganic N fertilizer under flooded condition. Although basal soil respiration rate was higher in the rice straw compost treatment, qCO2 was significantly lower in the rice straw and rice straw compost treatments as compared with the control in the –N

Table 6.11 Total C and N applied to the soil in 40 years and percent retained in the soil (Fukuoka LTE, 2003).

Treatment	Total C applied in 40 yrs kg ha^{-1}	Increase in C[a] mg g^{-1}	increase in C[b] kg ha^{-1}	C retained in the soil %	Total N applied in 40 yrs kg ha^{-1}	Increase in N[c] mg g^{-1}	increase in N[b] kg ha^{-1}	N retained in the soil %
Rice straw	139200	4.7	9300	6.7	2584	0.42	840	32.5
Rice straw compost	73840	8.7	17300	23.4	2232	0.80	1600	71.7
Rye grass / Wheat straw	110592	4.8	9600	8.7	2950	0.43	850	28.8

[a] computed as: C in soil with organic residue - C in control soil; average from -N and +N treatments

[b] bulk density = 1 g cm^{-3}

[c] computed as: N in soil with organic residue - N in control soil; average from -N and +N treatments

Table 6.12 Effect of long-term organic matter treatment on MBC under aerobic and flooded conditions (Fukuoka LTE, 2003).

Treatment	MBC (mg g^{-1} dry soil)							MBC (% of total C)						
	50% WFPS				flooded			50% WFPS				flooded		
	-N	+N	mean		-N	+N	mean	-N	+N	mean		-N	+N	mean
Control	0.71	0.83	0.77	b	0.64	0.59	0.61 b	3.6	3.9	3.7	a	3.2	2.7	3.0 a
Rice Straw	1.08	1.07	1.07	a	0.67	0.74	0.71 ab	4.2	4.0	4.2	a	2.7	2.9	2.8 a
Rice Straw Compost	1.21	1.20	1.20	a	0.84	0.85	0.85 a	4.2	4.0	4.1	a	2.9	2.8	2.9 a
Rye grass/Wheat Straw	1.04	1.09	1.07	a	0.79	0.85	0.82 ab	4.1	4.2	4.2	a	3.2	3.3	3.2 a
Mean	1.01	1.05			0.70	0.76		4.1	4.1			3.0	2.9	

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

Table 6.13 Effect of long term organic matter treatment on basal soil respiration and qCO_2 under aerobic and flooded conditions (Fukuoka LTE, 2003).

Treatment	Basal soil respiration (mg C kg^{-1} dry soil day^{-1})									qCO_2 (μg CO_2-C mg^{-1} MBC hr^{-1})								
	50% WFPS				flooded					50% WFPS				flooded				
	-N	+N	mean		-N	+N	mean			-N	+N	mean		-N	+N	mean		
Control	9.7	8.6	9.2	b	28.0	13.7	20.9	a		0.57 a	0.36 a*	0.46		1.85	0.99	1.42	a	
Rice Straw	11.4	11.6	11.5	ab	15.6	17.8	16.7	a		0.44 b	0.46 a	0.45		0.93	1.08	1.00	a	
Rice Straw Compost	12.4	12.4	12.4	a	26.6	9.1	17.9	a		0.43 b	0.44 a	0.43		1.36	0.46	0.91	a	
Rye Grass/Wheat Straw	11.4	11.4	11.4	ab	14.0	14.1	14.0	a		0.46 ab	0.45 a	0.45		0.71	0.69	0.78	a	
Mean	11.2	11.0			21.0	13.7	*			0.47	0.42			1.25	0.80	*		

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

Table 6.14 Flush of CO_2 3 days after rewetting of dried soil as affected by organic matter treatments (Fukuoka LTE, 2003).

Treatment	Flush of CO_2 aerobic mg C kg^{-1} dry soil				Flush of CO_2 anaerobic mg C kg^{-1} dry soil				Flush of CO_2 / HWEC aerobic μg C mg^{-1} HWEC hr^{-1}				Flush of CO_2 / HWEC anaerobic μg C mg^{-1} HWEC hr^{-1}			
	-N	+N	mean		-N	+N	mean		-N	+N	mean		-N	+N	mean	
Control	417	408	412	b	44.7 b	47.4 c	46.1		15.5	12.9	14.2	a	5.0	4.5	4.8	a
Rice Straw	440	444	442	a	53.3 ab	57.3 b	55.3		10.9	10.7	10.8	b	4.0	4.2	4.1	a
Rice Straw Compost	440	431	435	a	60.1 a	77.6 a*	68.8		10.6	9.0	9.8	b	4.4	4.9	4.6	a
Wheat Straw	424	449	437	a	60.8 a	69.8 a*	65.3		10.0	10.9	10.4	b	4.3	5.1	4.7	a
Mean	430	433			54.7	63.0 *			11.7	10.9			4.4	4.6		

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

treatment under aerobic condition (Table 6.13) indicating an efficient utilization of C by microorganisms. Addition of inorganic N reduced the qCO_2 in the control but not in the organic residue treatments. Thus, qCO_2 from treatments with organic residues did not vary significantly from the control in the presence of inorganic N fertilizer under aerobic condition. Under flooded condition, qCO_2 did not vary significantly among the different organic residue treatments but inorganic N treatment reduced qCO_2 significantly.

6.5.2.4 Three-day flush of CO_2 and hot water-extractable C

A flush of CO_2 was observed during the first 3d after rewetting of air-dried soil to 50% WFPS and after flooding. Under aerobic and flooded conditions, this was significantly greater in the residue-treated soils than in the control. Under flooded condition the 3d flush of CO_2 was significantly higher in the rice straw compost and wheat straw treatments than in the rice straw treatment (Table 6.14). Inorganic N application also increased the flush of CO_2 3d after rewetting under flooded condition.

All the organic matter treatments showed a significant increase in HWEC over the control but did not differ from one another. However, when expressed as a percentage of total C, only the wheat straw treatment differed significantly from the control. The HWEC fraction was not affected by inorganic N application (Table 6.15).

6.5.2.5 Potential mineralizable N

Positive effects of organic residue treatment were also observed in terms of PMN measured 1 month after incubation in both aerobic and flooded conditions (Table 6.16). Under aerobic condition, no significant differences were observed among the 3 organic residue treatments. However, under flooded condition, composted rice straw significantly increased PMN over that of rice or rye grass / wheat straw.

6.5.2.6 Permanganate-oxidizable C

Permanganate-oxidizable C comprises a larger fraction of the SOM pool than the more labile fractions - HWEC and MBC (section 6.5.1). It has also been used as a basis for the development of a C management index used to evaluate soil management practices (Blair et al. 1997). The MnOC measured from the organic residue treatments were all significantly higher than the control. The rice straw

Table 6.15. Effect of long-term organic matter treatment on HWEC (Fukuoka LTE, 2003).

Treatment	HWEC (mg g^{-1} dry soil)				HWEC (% of total C)			
	-N	+N	mean		-N	+N	mean	
Control	0.38	0.44	0.41	b	1.92	2.05	1.99	b
Rice Straw	0.56	0.58	0.57	a	2.27	2.24	2.25	ab
Rice Straw Compost	0.58	0.67	0.62	a	2.02	2.22	2.12	ab
Wheat Straw	0.59	0.58	0.58	a	2.36	2.24	2.30	a
Mean	0.53	0.57			2.14	2.19		

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

Table 6.16. Effect of organic matter treatment on PMN under aerobic and flooded conditions (Fukuoka LTE, 2003).

Treatment	PMN (mg kg^{-1} dry soil)								
	50% water-filled pore space				flooded				
	-N	+N	mean		-N		+N		mean
Control	50.1	46.9	48.5	b	51	b	53	d	52
Rice Straw	58.6	66.2	62.4	a	105	a	101	b	103
Rice Straw Compost	61.1	66.6	63.9	a	103	a	121	a*	112
Wheat Straw	58.3	64.1	61.2	a	99	a	80	c*	89
Mean	57.0	61.0 *			89		89		

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

Table 6.17. Effect of organic matter treatment on soil MnOC (Fukuoka LTE, 2003).

Treatment	MnOC (mg g^{-1} dry soil)				MnOC (% of tot C)			
	-N	+N	mean		-N	+N	mean	
Control	2.92	3.05	2.99	c	14.67	14.23	14.45	c
Rice Straw	3.99	4.03	4.01	b	16.03	15.68	15.86	b
Rice Straw Compost	4.88	5.11	5.00	a	17.14	16.99	17.06	a
Wheat Straw	3.91	3.77	3.84	b	15.59	14.62	15.11	bc
Mean	3.93	3.99			15.86	15.38		

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

compost treatment had a significantly higher MnOC than the rice and rye grass / wheat straw treatments. The MnOC fraction of total soil C also increased significantly with the incorporation of rice straw and rice straw compost indicating the accumulation of MnOC from the added plant residues (Table 6.17). The measured

MnOC may include labile C together with more resistant lignin-like structures with hydroxyl groups that are readily oxidized by permanganate.

6.5.2.7 Rice grain yields and N uptake

No significant rice yield trends were observed in all the organic matter treatments including the control from 1989 to 2003 (Fig. 6.9) indicating that rice yields were

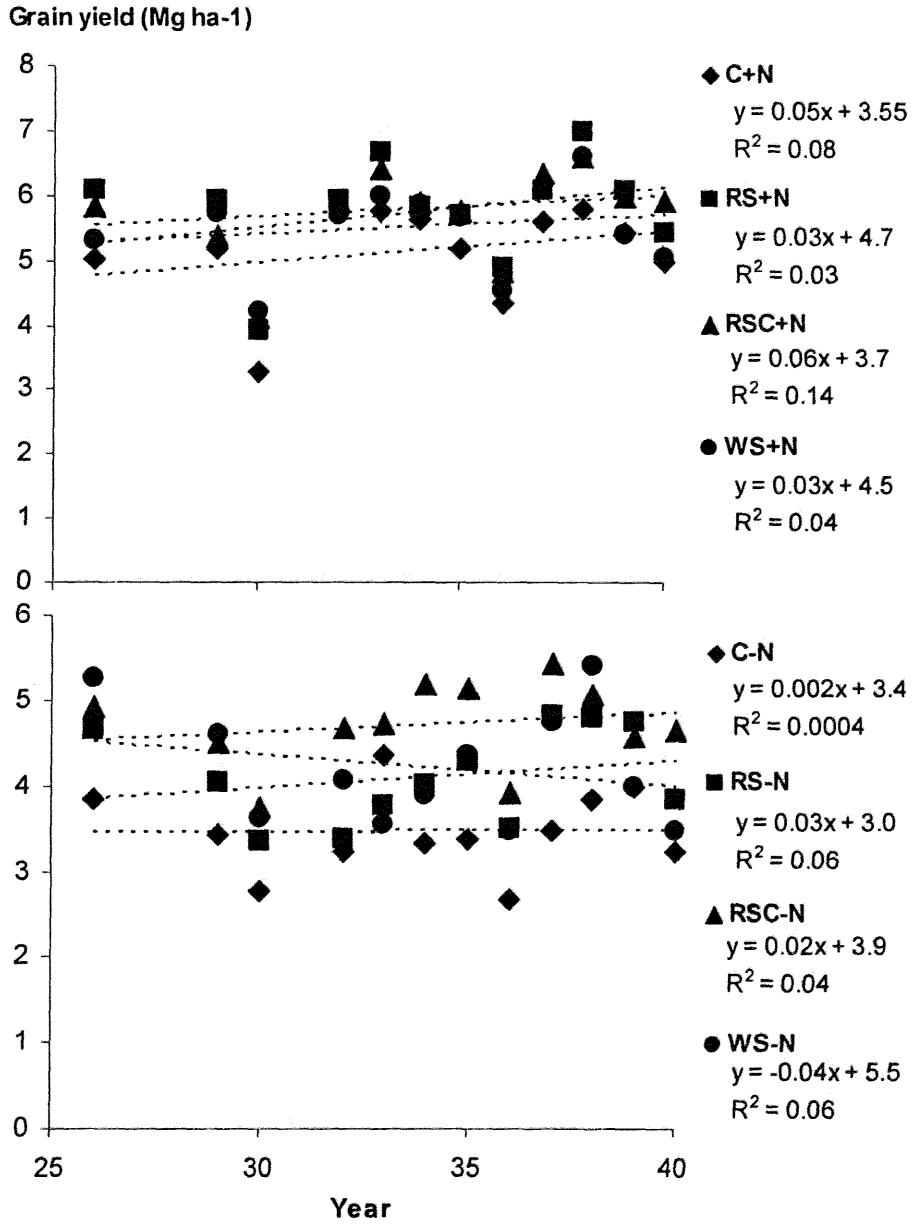


Fig. 6.9 Rice grain yield trends as affected by inorganic and organic fertilization in Fukuoka, Japan.

being maintained or were stable even without fertilization. However, a 3-way analysis of variance (organic matter x inorganic N x year) showed a highly significant ($P<0.01$) effect of organic matter and inorganic N treatments on grain yield and N uptake. Mean comparison by the Tukey test showed that all the organic matter treatments significantly improved rice yields and N uptake as compared with the control (Table 6.18). Moreover significantly higher rice yields and N uptake were obtained from the incorporation of composted rice straw than from uncomposted rice straw. In treatments without inorganic N fertilizer, grain yield was significantly correlated with total organic C, HWEC, MBC (at 50% WFPS), basal soil respiration (at 50% WFPS), and PMN (Fig.6.10).

Table 6.18 Effect of long-term organic matter treatment on grain yield and plant N uptake (Fukuoka LTE, 2003).

Treatment	N uptake (kg ha^{-1})				Grain Yield (Mg ha^{-1})			
	-N	+N	mean		-N	+N	mean	
Control	58	100	79	c	3.5	5.1	4.3	c
Rice Straw	73	118	95	b	4.1	5.7	4.9	b
Rice Straw Compost	83	120	102	a	4.7	5.7	5.2	a
Wheat Straw	77	113	95	b	4.2	5.5	4.9	b
Mean	73	113	*		4.1	5.5	*	

In a column, means followed by a common letter are not significantly different at the 5% level by the Tukey test. If treatment x N interaction is significant, means within each N are compared. Otherwise, means of −N and +N are compared.

6.5.3 Long-term effects of organic and inorganic fertilization on soil parameters and soil quality in rice-wheat systems in India and Nepal

6.5.3.1 Cumulative effects of combinations of inorganic and organic amendments on chemical and microbiological soil properties

6.5.3.1.1 Soil particle size, pH, CEC, available P and K

The soil texture remained unchanged with long-term treatments including organic amendments in the two soils. However, the difference in texture between the two soils (loamy sand in Ludhiana and silty loam in Bhairahawa) provided opportunity to assess changes in soil properties in relation to texture. Soil pH did not change with various organic and nutrient amendments at both sites except with the combination of full NPK+GM (LT 5), in which soil pH decreased by 0.3 to 0.9 units compared with the other treatments in Ludhiana. In Bhairahawa, soil pH increased by 0.2 units on average with the addition of P but it was not affected by

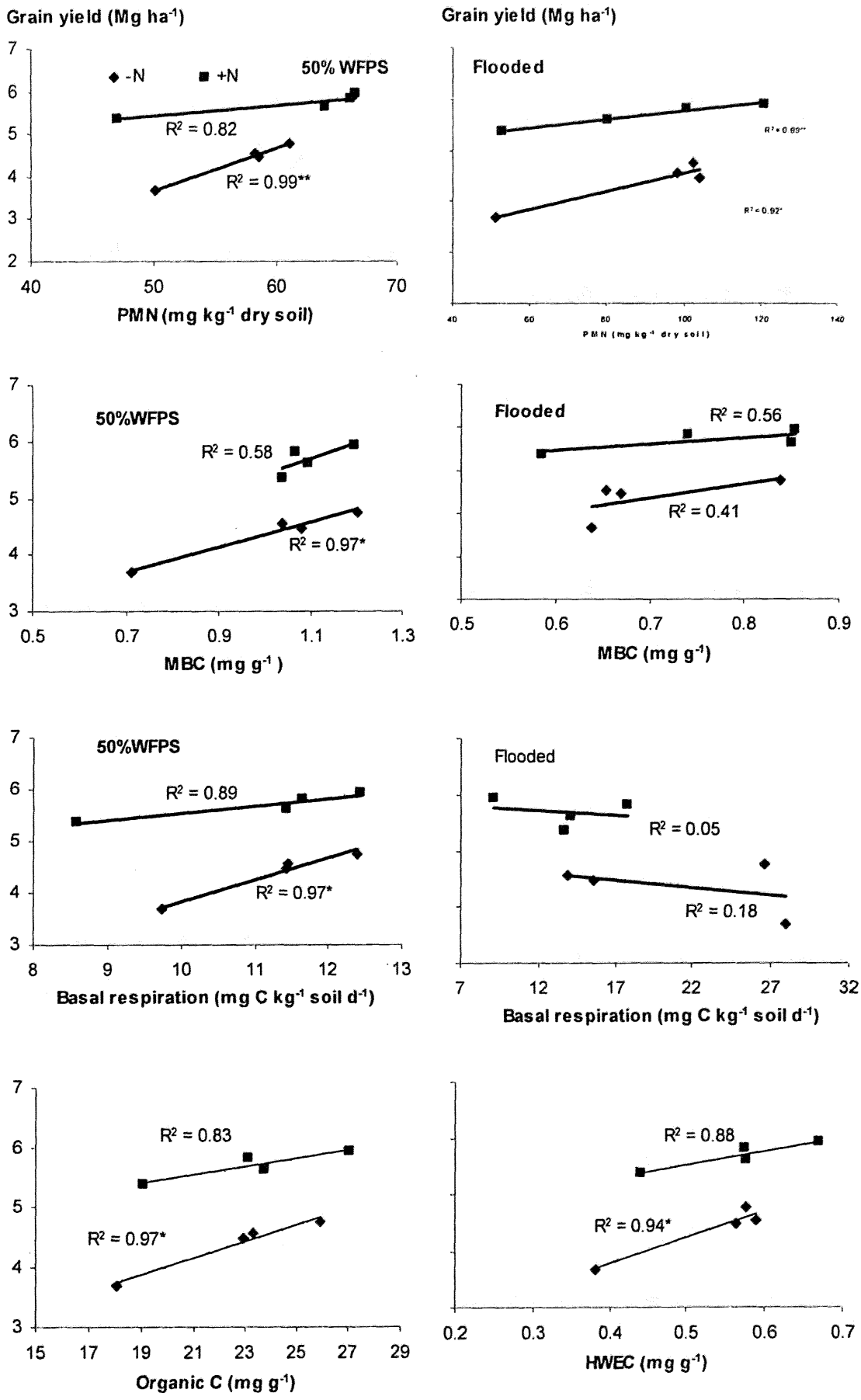


Fig. 6.10 Relationships of grain yield with total organic C, hot water-extractable C, microbial biomass C and potential mineralizable N (Fukuoka, Japan).

organic treatments (Table 6.19). Cation-exchange capacity remained unchanged in Ludhiana, but, in Bhairahawa, FYM (BT 3) increased CEC by 0.5 to 0.7 units compared with inorganic N (BT1) and GM (BT2) treatments. The addition of P increased CEC by 0.6 units. As expected, FYM amendments showed a remarkable increase in Olsen soil P in Ludhiana, where it increased by 477% (Table 6.19). The amount of organic P added from FYM in Ludhiana was even greater than the amount of inorganic P added (Table 6.3). In Bhairahawa, the averages of treatments with and without P showed a significant P increase of 33% due to FYM (Table 6.19). Results show that there was a greater accumulation of P in Ludhiana than in Bhairahawa although more P (inorganic organic) was being added in Bhairahawa than in Ludhiana. It should be noted that, in Ludhiana, FYM was added to rice while in Bhairahawa it was added to wheat. Available K, which was less than 3 mg 100g-1 at both sites remained unchanged (data not shown).

6.5.3.1.2 Soil C

FYM amendment (LT3 and BT3) compared to the control (LT1 and BT1) increased total organic C and MnOC fractions by 32% and 70%, respectively, but the GM and WS (LT4 and BT2) did not change significantly (Table 6.19). In Bhairahawa, total organic C with GM+NP (BT2+P) and FYM+NP (BT3+P) were 26-32% and 16-24% higher than those with only N (BT1-P) and NP (BT1+P), respectively, while that with GM+N (BT2-P) was even lower by 9% than with N only (BT1-P) (Table 6.19). The low soil C without P was due to the incorporation of a suboptimal amount of GM biomass as compared to that in the GM+NP treatment (BT2+P), where P addition enhanced biomass production.

The soil MnOC fraction was higher in the FYM treatment (LT3) than in any of the other treatments in Ludhiana. Farmyard manure was earlier reported to contain higher MnOC and lignin content than GM (Table 6.). The HWEC had treatment differences similar to those of total C in Ludhiana but not in Bhairahawa, where no treatment differences were observed (Table 6.19). At both sites, organic manures did not show a significant positive effect on MBC (Table 6.20).

6.5.3.1.3 Total N and PMN

Farmyard manure (LT3 and BT3) increased total soil N by 40% and 14% in Ludhiana and Bhairahawa, respectively. Similarly, total mineralized N measured

Table 6.19 Effects of organic and inorganic fertilization on soil properties measured from air-dried soil (Ludhiana and Bhairahawa LTE, 2003)

Treatment	pH 1:1 (H_2O)	CEC meq $100g^{-1}$	Olsen P mg $100g^{-1}$	Tot C	Org C	MnOC	HWEC	Tot N	Min N	DA ug TPF g^{-1} soil
				mg g^{-1}						
Ludhiana										
LT1	7.4 a††	5.8	0.4 b	4.53 b	4.53 b	0.73 b	0.18 b	0.52 b	8.07 b	46.5 b
LT2	7.0 ab	6.3	0.9 b	4.78 ab	4.78 ab	0.81 b	0.17 b	0.54 b	14.15 b	39.8 b
LT3	7.1 ab	6.8	5.2 a	6.48 a	6.00 a	1.24 a	0.25 a	0.73 a	32.85 a	69.7 a
LT4	6.8 ab	6.0	0.7 b	5.37 ab	5.28 ab	0.94 b	0.21 ab	0.59 ab	13.84 b	50.2 b
LT5	6.5 b	6.4	1.1 b	5.37 ab	4.94 ab	0.94 b	0.22 ab	0.62 ab	23.14 ab	45.7 b
Bhairahawa										
Organic fertilizer x P means										
BT1-P	8.0	7.7 b	0.2	8.44 bc	7.44 b	1.34	0.29	0.89 bc	10.08	95.8
BT2-P	8.0	7.5 b	0.2	7.88 c	6.76 c	1.35	0.27	0.82 c	12.10	83.1
BT3-P	7.9	8.5 a	0.5	9.68 ab	9.41 a	1.68	0.31	1.00 ab	13.25	115.2
BT1+P	7.8	8.1 ab	2.1	9.08 abc	8.03 b	1.38	0.28	0.96 abc	10.95	153.6
BT2+P	7.8	8.7 a	1.8	10.21 a	9.35 a	1.60	0.33	1.07 a	11.00	168.2
BT3+P	7.8	8.6 a	2.8	10.33 a	9.80 a	1.90	0.30	1.11 a	12.39	176.4
Organic fertilizer means										
BT1	7.9	7.9 b	1.2 b	8.76	7.73	1.36 b	0.29	0.93	10.52	124.7
BT2	7.9	8.1 b	1.0 b	9.05	8.06	1.48 ab	0.30	0.94	11.56	125.7
BT3	7.8	8.6 a	1.6 a	10.00	9.61	1.79 a	0.31	1.05	16.48	145.8
P means										
-P	8.0 a	7.9 b	0.3 b	8.67	7.87	1.46	0.33	0.90	14.25	98.0 b
+P	7.8 b	8.5 a	2.2 a	9.87	9.06	1.62	0.33	1.04	11.45	166.1 a

†MnOC, permanganate oxidizable C; HWEC, hot water-extractable C; TPF, triphenyl formazan; DA, dehydrogenase activity

†† Comparison of means is given where F values from analysis of variance are significant. In a column, means followed by a common letter are not significantly diffrerent from each other at the 5% level by the Tukey test.

Table 6.20 Effects of organic and inorganic fertilization on soil properties measured from incubated soil (Ludhiana and Bhairahawa LTE, 2003)†

Treatment	MBC		Basal soil respiration		q CO_2		3d CO_2		PMN	
	50% WFPS	flooded	50%WFPS	flooded	50%WFPS	flooded	50%WFPS	flooded	50% WFPS	flooded
	mg C g^{-1} soil		mg C kg^{-1} soil d^{-1}		µg C mg^{-1} MBC h^{-1}		mg C kg^{-1} soil d^{-1}		mg N kg^{-1} soil	
Ludhiana										
LT1	0.22 a††	0.23	13.14	10.09	2.67 ab	4.40	55.98	10.20 ab	17.26 b	2.56 b
LT2	0.22 a	0.31	9.97	10.56	2.01 b	3.27	54.27	8.54 b	16.52 b	2.03 b
LT3	0.20 ab	0.37	9.18	13.67	1.98 b	3.45	67.81	11.63 ab	25.01 ab	3.85 ab
LT4	0.11 ab	0.27	10.12	12.08	3.98 ab	4.23	65.97	12.93 ab	25.62 ab	6.26 a
LT5	0.10 b	0.35	8.77	17.30	6.11 a	4.67	62.75	17.83 a	29.74 a	5.10 ab
Bhairhawa										
Organic fertilizer x P means										
BT1-P	0.34	0.38	15.19	12.02	1.83	1.33	81.33	9.63	31.19	8.31
BT2-P	0.33	0.31	14.84	12.08	1.92	1.65	71.67	8.08	27.64	10.19
BT3-P	0.27	0.30	14.34	14.78	2.82	2.20	84.18	9.66	33.74	12.11
BT1+P	0.30	0.37	15.63	15.89	2.20	1.80	74.53	12.39	33.57	10.17
BT2+P	0.29	0.33	14.84	13.66	2.47	1.81	76.43	9.18	34.18	12.32
BT3+P	0.27	0.28	15.22	13.25	2.64	2.25	89.62	12.99	38.63	14.43
Organic fertilizer means										
BT1	0.32	0.38	15.34	13.96	2.02	1.56	77.93 ab	11.01	32.38	9.24 b
BT2	0.31	0.32	15.34	13.96	2.19	1.73	74.05 b	8.63	30.91	11.26 ab
BT3	0.27	0.29	14.84	12.87	2.73	2.23	86.90 a	11.33	36.19	13.27 a
P means										
-P	0.32	0.33	14.80	13.00	2.20	1.70	79.10	9.10	30.90 b	10.20 b
+P	0.29	0.33	15.00	14.30	2.40	2.00	80.20	11.50	35.50 a	12.30 a

† MBC, microbial biomass C; PMN, potential mineralizable N; WFPS, water-filled pore space

†† Comparison of means is given where F values from analysis of variance are significant. In a column, means followed by a common letter are not significantly diffrerent from each other at the 5% level by the Tukey test.

from air-dried soil was highest with FYM (LT3) in Ludhiana (Table 6.19), but it did not vary among treatments in Bhairahawa. On the other hand, PMN had a variable response. In Ludhiana, it was highest with GM (LT5) under aerobic incubation and with WS under flooding (Table 6.20). In Bhairahawa, P application increased PMN under both incubations. In addition, compared to the control, PMN with FYM was 44% higher when soil was incubated under flooded conditions (Table 6.20).

6.5.3.1.4 Dehydrogenase activity

An organic source (FYM) increased DA measured from air-dried soil in Ludhiana but had no effect in Bhairahawa, though long-term addition of P had a stimulatory effect at the latter site (Table 6.19). The other treatments in Ludhiana did not show a significant effect in terms of DA. Since air-dried soils were used, these results can be used as indicators of long-term biological activity as measured from the activity of accumulated enzymes complexed in the soil matrix. Freshly collected soils, on the other hand, would show enzyme activity stimulated by recent soil amendments (Tabatabai, 1982).

6.5.3.1.5 Basal soil respiration, qCO_2, and flush of CO_2 following rewetting of dried soil

No differences in basal soil respiration were observed among treatments at both sites (Table 6.20). In Ludhiana, however, compared with NPK (LT2), the GM treatment (LT5) had a 2-fold increase in the 3-d flush of CO2 following rewetting of dried soil. The metabolic quotient (qCO_2 = basal respiration/MBC) which is inversely related to metabolic efficiency, was not different among treatments under flooded conditions. But qCO_2 increased 3 times in the GM treatment (LT5) relative to the NPK (LT2) and FYM (LT3) treatments under aerobic conditions. High qCO_2 values indicate that, during C mineralization of organic matter, microbes divert more C to respiration than to new microbial biomass, causing more C loss. On the other hand, a decline in qCO_2 may indicate the presence of microbial populations that are more efficient in incorporating C compounds into microbial cells, or availability of less labile organic residues. In Bhairahawa, the 3-d flush of CO2 after rewetting dried soil was highest with FYM

(BT3) and lowest with GM (BT2) under aerobic conditions (Table 6.20). No differences among treatments were observed under flooded conditions.

6.5.3.1.5 Accumulation of C and N from organic manures

Accumulations of soil C and N from organic amendments (LT3, LT4, and LT5) were estimated from the difference between unfertilized and fertilized treatments. In Ludhiana, the average accumulation of C as a fraction of the total organic C applied was 3 times higher with FYM than with WS and GM (Table 6.21). Similarly, the accumulation of N as a fraction of the total organic N applied was about 2 times higher with FYM than with GM and WS (Table 6.22). In Bhairahawa, increases in C over the N-fertilized and NP-fertilized controls were significant in both the GM (BT2+P) and FYM (BT3+P) treatments (Table 6.19) and the estimated %C accumulation as a fraction of C applied was similar in the two treatments (Table 6.21). Although the increase in soil N with FYM+NP (BT3+P) over the N treatment (BT1-P) was slightly higher than that with GM+NP(BT2+P), the %N accumulation as a fraction of the organic N applied was higher with GM+NP due to the lower amount of N applied from GM (Table 6.22). These estimates, however, do not account for C and N added as root biomass.

6.5.3.2 Time trends of key soil parameters as affected by inorganic and organic amendments

6.5.3.2.1 Total C, HWEC, and MnOC

In Ludhiana, analysis of variance (ANOV) over time showed a significant effect of year and treatment on total soil C and a significant treatment x year interaction. An earlier report by Bhandari et al. (2002), showed an increasing trend of total C only in the FYM+NPK (LT3) treatment up to the 15th yr. This study shows that total C continued to increase up to the 20th yr with FYM (LT3) together with the WS+NPK (LT5) treatment although total C increased at a slower rate in LT5. Total C remained stable in the other treatments (Fig. 6.11a). In Bhairahawa, no significant change in total soil C from the 3rd to the 15th year was observed (Fig. 6.11b).

On the other hand, analyses of variance and regression showed that HWEC declined significantly by 46-63% from the 5th to the 20th year in all except the FYM treatment (Fig. 6.12a). In Bhairahawa, ANOV also showed that HWEC

Table 6.21 Total amounts of C accumulated in the soil from continuous application of organic fertilizer (Ludhiana and Bhairahawa LTE, 2003).

Site	Treatment	C content of organic fertilizer (g kg^{-1})	Rate of application of organic fertilizer (Mg ha^{-1})	C:N (mole ratio)	Total C applied per year (kg ha^{-1})	No. of years applied	Total C applied (kg ha^{-1})	Increase in organic C relative to the control† (kg ha^{-1})	C accumulation as a fraction of total organic C applied %
Ludhiana, India	GM+NPK	376	2.86	21	1075	20	21507	855	4.0
	FYM+NPK	243	6	28	1458	20	29160	3233	11.1
	WS+NPK	404	6	94	2424	20	48480	1620	3.3
Bhairahawa, Nepal	GM+NP	450	3	26	1287	15	19305	4017	20.8
	FYM+NP	365	4	21	1460	15	21900	4956	22.6

† Increase in soil C was measured relative to an unfertilized control in Ludhiana and relative to an N-fertilized control in Bhairahawa.

Table 6.22 Total amounts of N accumulated in the soil from continuous application of organic fertilizer (Ludhiana and Bhairahawa LTE, 2003).

Site	Treatment	N content of organic fertilizer (g kg^{-1})	Rate of application (Mg ha^{-1})	C:N (mole ratio)	Organic N applied per year	No. of years applied	Total organic N applied (kg ha^{-1}	Increase in soil N relative to the control†	N accumulation as a fraction of organic N applied %
Ludhiana, India	GM+NPK	21	2.86	21	60	20	1200	225	18.8
	FYM+NPK	10	6	28	60	20	1200	466	38.8
	WS+NPK	5	6	94	30	20	600	144	24.0
Bhairahawa, Nepal	GM+NP	20	3	26	60	15	900	365	40.6
	FYM+NP	20	4	21	80	15	1200	449	37.4

† Increase in soil N was measured relative to an unfertilized control in Ludhiana and relative to an N-fertilized control in Bhairahawa.

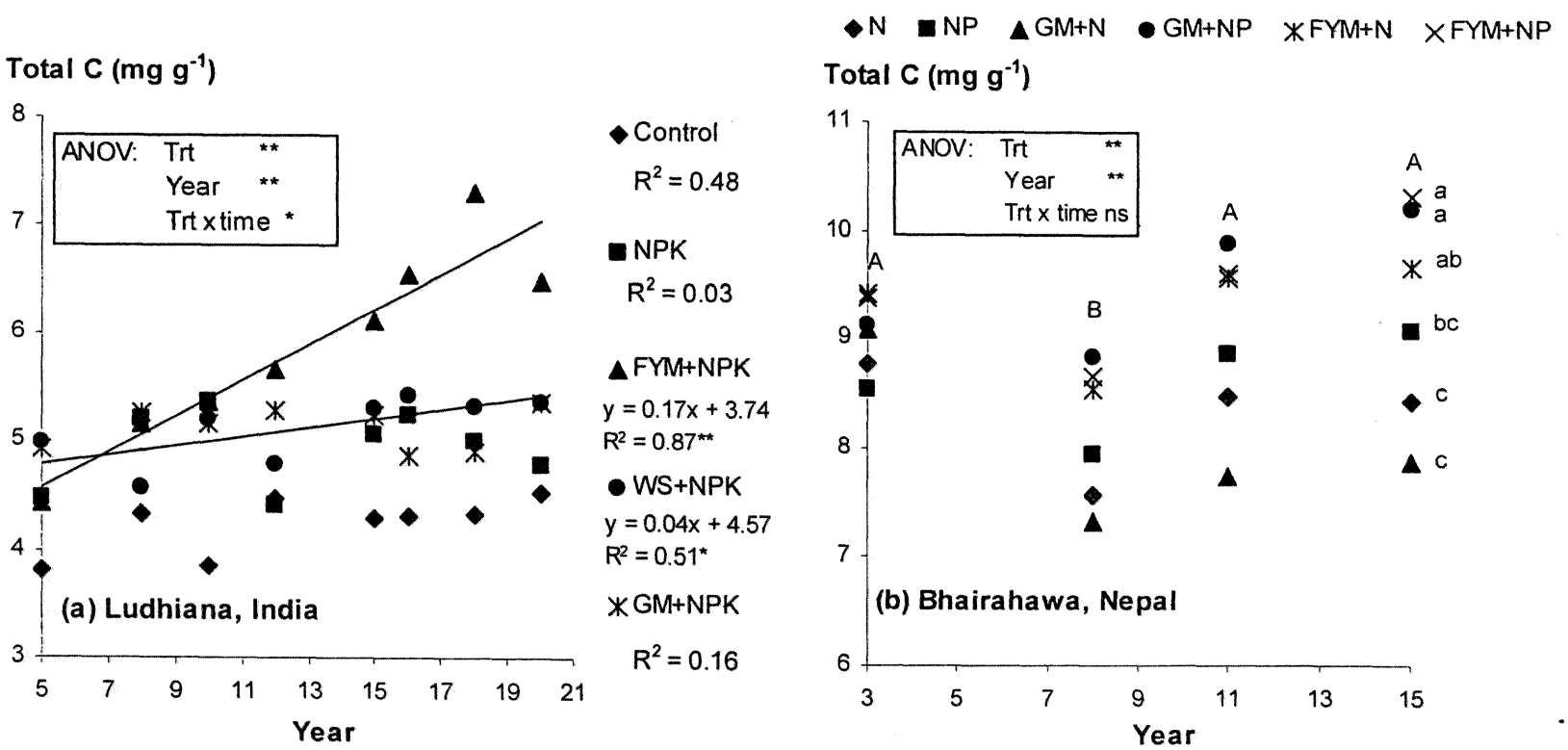


Fig. 6.11 Changes in soil total C as affected by organic and inorganic fertilization in Ludhiana (a) and Bhairahawa (b). In Bhairahawa, uppercase letters indicate mean comparison among years averaged over treatments while lowercase letters indicate mean comparison among treatments averaged over years. Means with a common letter are not significantly different from each other at the 5% level by the Tukey test.

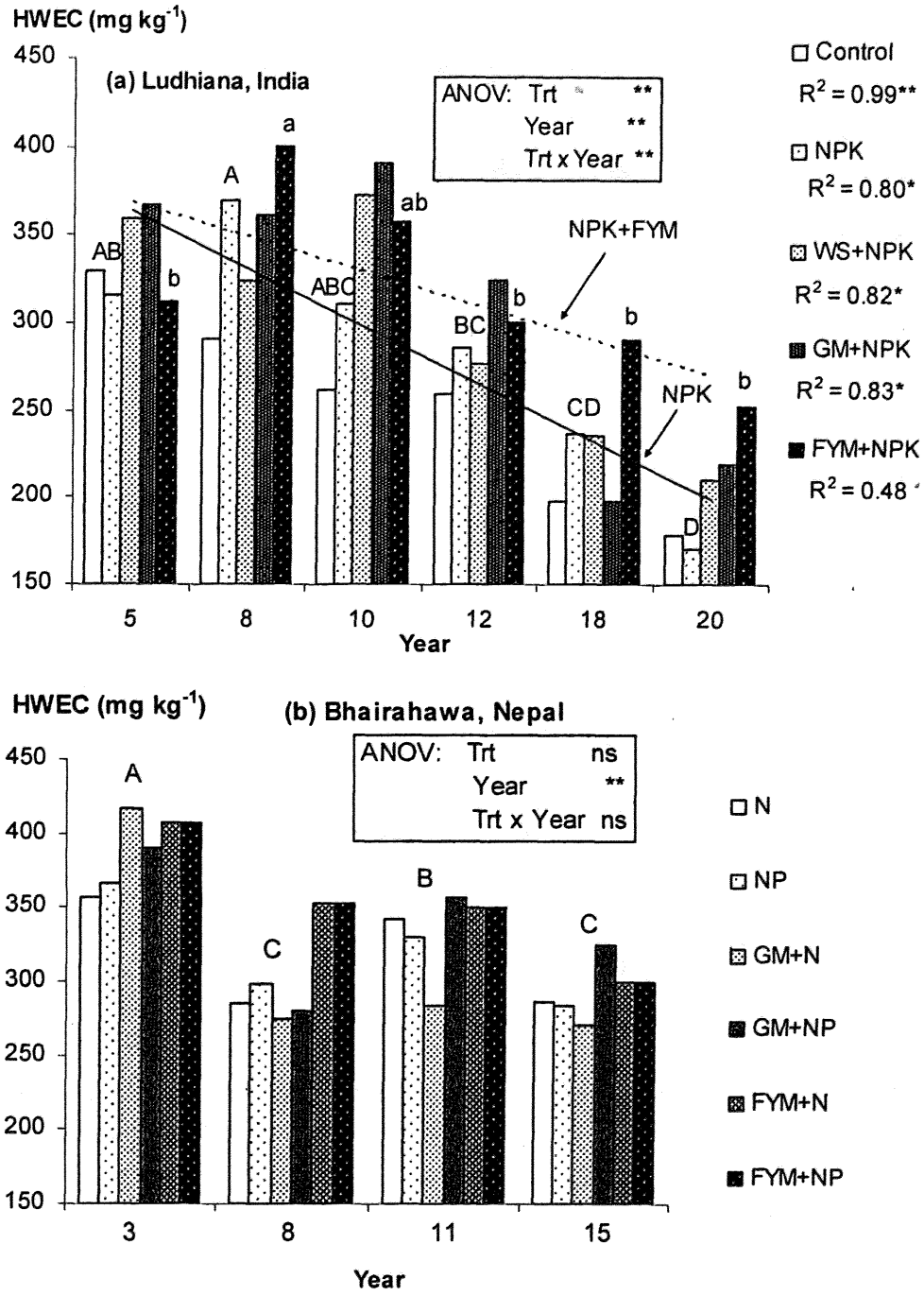


Fig. 6.12 Changes in HWEC as affected by organic and inorganic fertilization in Ludhiana (a) and Bhairahawa (b). In (a), uppercase letters indicate mean comparison among years within NPK treatment while lower case letters indicate mean comparison among years within FYM+NPK treatment. Regression lines are for NPK (solid line) and FYM (broken line). In (b), letters indicate mean comparison among years averaged over treatments. Means with a common letter are not significantly different from each other at the 5% level by the Tukey test.

declined by 25% on average from the 3rd to the 15th year but there was no year x treatment interaction (Fig. 6.12b).

The time trend of the MnOC fraction in Ludhiana was similar to that of total C but opposite to that of HWEC. The MnOC showed an increasing trend up to the 15th year only in the FYM+NPK (LT3) treatment. (Fig. 6.13). The observed opposite trends of MnOC and HWEC are understandable, as lignin, which is related to MnOC, would tend to accumulate with organic manure incorporation, whereas HWEC would be readily mineralized.

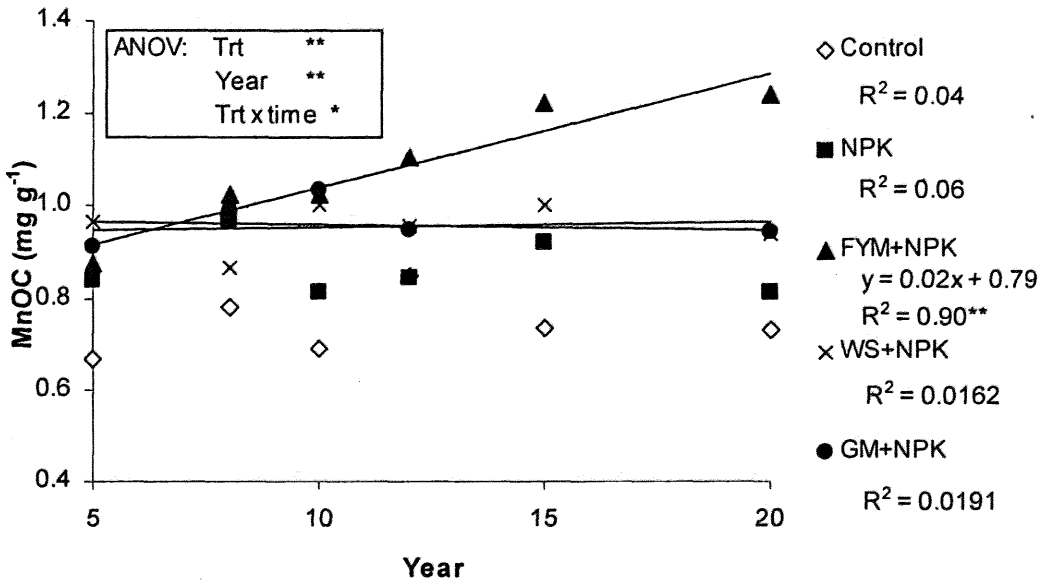


Fig. 6.13 Changes in MnOC over the years as affected by various organic and inorganic amendments (Ludhiana LTE).

6.5.3.2.2 Total N and PMN

Earlier reports (15 years' data) from the LTE at Ludhiana have shown significant declining trends in total soil N in the control, GM, and NPK treatments except for the FYM treatment (Bhandari et al., 2002). The present analysis (20 years' data) shows that total soil N has continued to decline in all treatments except the FYM treatment (Fig. 6.14a). Soil N decreased by 7-22% in all treatments except FYM+NPK (LT3). In Bhairahawa, the FYM+NP (BT3+P) treatment showed an increasing soil N content, whereas the N (BT1-P) and GM+N (BT2-P) treatments showed declining trends with time. However, due to the limited number of data points, only the GM+N treatment gave a significant ($P<0.05$) regression coefficient (Fig. 6.14b). The PMN from archived soil samples

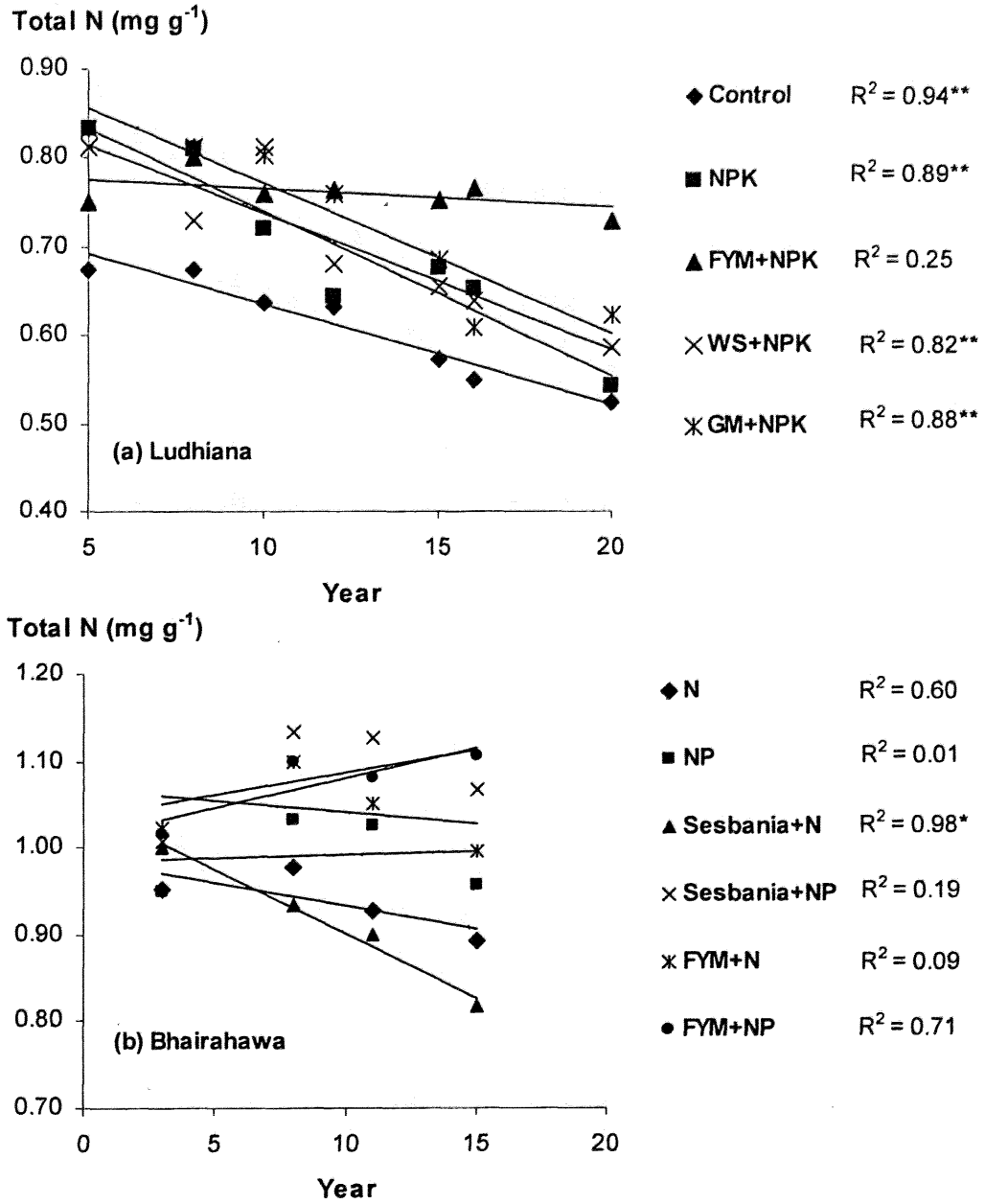


Fig. 6.14 Changes in total soil N over 20 years as affected by inorganic and organic fertilizer treatments (Ludhiana LTE).

in Ludhiana showed a decrease of 73-91% in all treatments from the 5th to the 20th year (Fig. 6.15). This decline in PMN may be related to the decrease in the decomposable organic matter as shown by the high correlation between PMN and HWEC across years (Fig. 6.16). An accumulation of more stable organic matter over the years may have led to a slower release of mineralizable N.

6.5.3.2.3 Dehydrogenase activity

Dehydrogenase activity from archived soil samples in the Ludhiana LTE showed increasing trends starting from the 5th yr in all treatments except for the

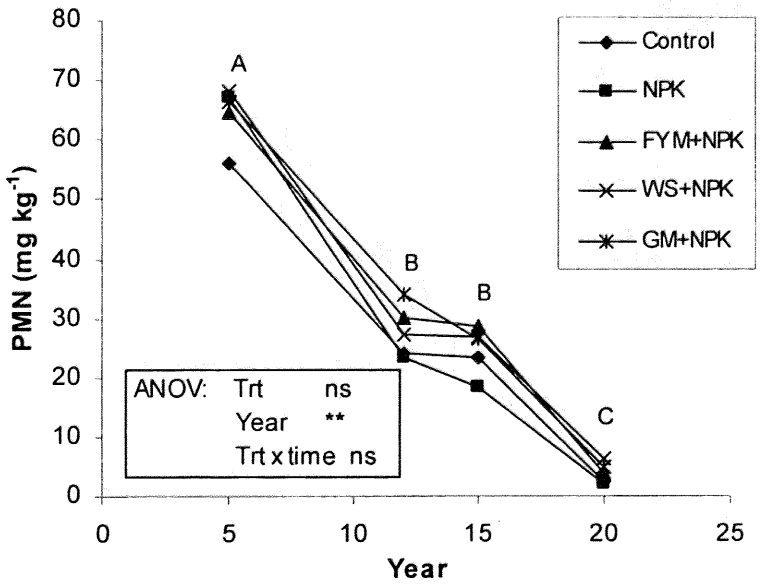


Fig. 6.15 Changes in PMN across years as affected by various organic and inorganic amendments. Uppercase letters above the points indicate comparison among year means. Means with a common letter are not significantly different at the 5% level by the Tukey test.

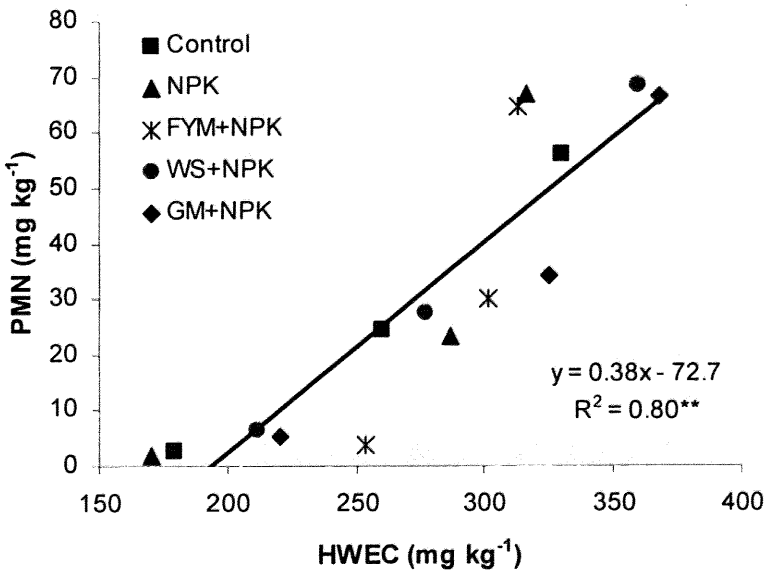


Fig. 6.16 Relationship between PMN and HWEC across years in Ludhiana, India.

GM+NPK (LT5) treatment, which already showed a significantly higher activity than the control in the 5th year (Fig. 6.17). However, the FYM+NPK (LT3) treatment increased by 488% over the control and showed the highest activity in the 15th year. Only LT3 had an increased DA over the control after 20 years, which is also an indication of enhanced biological activity over a long term. On

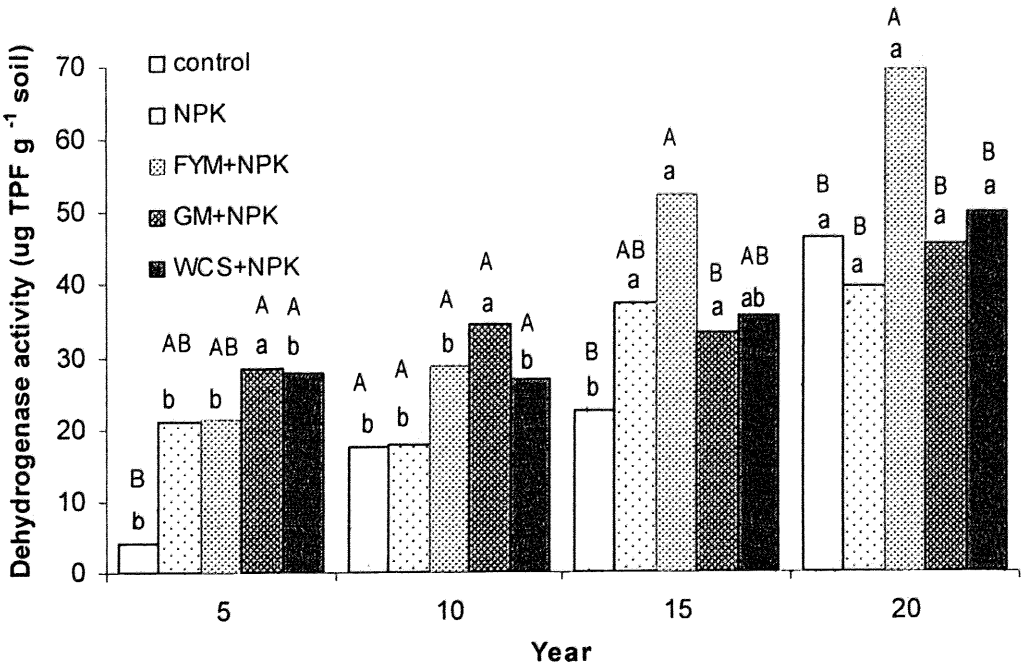


Fig. 6.17 Changes in soil dehydrogenase activity as affected by long-term organic matter treatments in a rice-wheat system in Ludhiana, India. Means within each year with a common uppercase letter and means across years within each treatment with a common lowercase letter are not significantly different at the 5% level by the Tukey test.

the other hand, GM exhibited a more immediate effect on DA. These results show the accumulation of dehydrogenase even in the control, most probably because of the accumulation of root residues. Dehydrogenase is involved in the oxidation of organic matter in soils. Thus, it was expected that the FYM treatment, which had the highest soil organic C, also exhibited the highest DA in the long term.

6.6 Discussion

6.6.1 Long-term effects of rice and wheat straw incorporation on soil parameters and soil quality in a rice-wheat system in Fukuoka, Japan

Results from the 40-yr LTE in Fukuoka, Japan, have demonstrated a significant increase in total soil organic C from the incorporation of rice straw, rye grass / wheat straw and rice straw compost, with no significant effect on the soil C:N ratio and no drastic change in the soil pH after 40 years. Greater C and N accumulation was observed from composted rice straw than from uncomposted rice or wheat straw indicating that composting plant residues before incorporation immobilizes C and slows down the C mineralization rate (Table 6.11). Bernal et al. (1998) found that C mineralization

decreased as the composting time lengthened and suggested that composting is the best way of obtaining maximum C accumulation. Data obtained from earlier years (Lowland Soil Management Lab., Dept. of Lowland Farming, Kyushu National Agricultural Experiment Station and Plant Environment Division, National Honam Agricultural Experiment Station, Rural Development Administration 1992; Tsuchiya et al. 2000) show that total soil C in the composted and uncomposted rice straw treatments were very similar after 14 years of cultivation although more C was being added as rice straw. Continuous application of rice straw compost increased total soil C from the 14th to the 28th year. After 28 years, the compost treatment showed no change in total C suggesting that a steady state has been reached. On the other hand, there was no change in the total soil C content of the rice straw treatment from the 14th to the 28th year but there was a decrease from the 28th to the 35th year indicating net C losses from this treatment. Consequently, the difference between the total C of the composted and uncomposted rice straw treatments increased after the 28th year. Apparently, there was a decrease in total soil N from the 28th to the 40th year in all the treatments but the magnitude was less with rice straw compost than with rice straw treatment (Fig. 6.18). The rice-wheat system may be prone to large N losses due to alternate soil wetting (flooded condition for rice) and drying (aerobic condition for wheat) (Kundu and Ladha, 1995). Thus, it is important to determine the optimum rate of compost application that would minimize N losses.

Organic matter incorporation improved the capacity of the soil to hold nutrients as shown by increased CEC with organic treatments. Plant N uptake was evidently higher in treatments with organic matter incorporation than without. Further, N uptake was significantly enhanced by composting rice straw before incorporation (Table 6.18) due to the decrease in the C:N ratio of rice straw after composting and increased N availability (Table 6.2).

Microbial biomass C and HWEC represent two of the more labile pools that make critical contributions to nutrient flows, organic matter turnover and structural stability of aggregates. Microbial biomass C is usually measured from freshly collected soil when monitoring short-term changes or seasonal changes. However, in this study, MBC was measured from air-dried soils that were incubated under aerobic and flooded conditions. MBC is expected to change after drying and re-wetting but the relative differences in MBC among treatments after undergoing similar drying and re-wetting processes could be indicative of long-term soil and agronomic management effects. Moreover, incubation experiments done in the laboratory under standardized conditions provide an estimate of

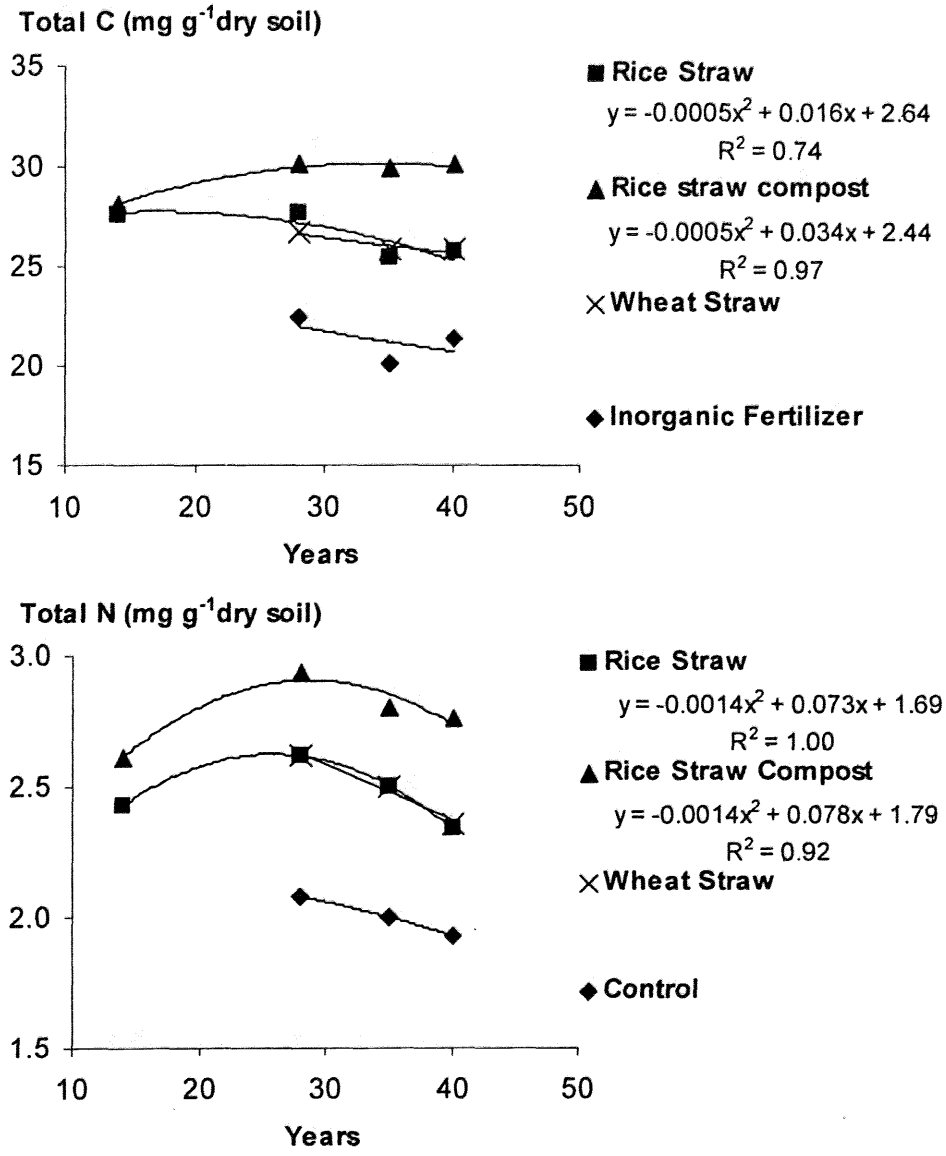


Fig. 6.18 Changes in total soil C and N under different organic fertilizer management in the LTE at Fukuoka, Japan. (No data points for the 14th yr are available for the control and wheat straw treatments)

the soil's potential. Franzluebbers et al. (1996) determined microbial biomass and nitrogen mineralization following rewetting of dried soil. They found that pre-incubation periods of one and 15 days prior to fumigation gave estimates of MBC using chloroform fumigation incubation (CFI) most similar to those determined on field-moist soil. They also found that soil MBC determined on field-moist and dried soil were both correlated with C mineralization during all incubation periods.

In this study, only the compost treatment significantly increased MBC as compared with the control under flooded condition. Even at the end of the breakdown process, compost still carries an enormous population of microorganisms. The application of compost not only adds much microbial life but supplies food for the microorganisms

already present in the soil since the amount of decomposable C (HWEC) also increased significantly over the control with compost treatment. In agreement with our results, Kanazawa and Hayano (1992) found that microbial biomass N and ATP measured by toluene treatment from the rhizosphere and non-rhizosphere soil of rice in 1991, in this LTE, were highest in the compost plot followed by the chemical fertilizer plot and lowest in the no fertilizer plot.

Measurements of basal soil respiration, MBC and the metabolic quotient (qCO_2) provide a method to relate both the size and activity of the microbial biomass. The qCO_2 has been used to study soil over time and, generally, the quotient decreases as the soil ages (Insam et al. 1988; Insam et al. 1989; Anderson et al. 1990). A high qCO_2 implies that microorganisms cannot metabolize C efficiently. A high quotient may also infer stress, an immature ecosystem or a more respirable substrate (Sparling 1997). The rice straw and rice straw compost treatments exhibited a lower qCO_2 as compared with the control under aerobic incubation, implying an efficient metabolism of C by microorganisms and an enhanced C accumulation in the soil.

Franzluebbers et al. (2000) suggested the use of the CO_2 pulse following wetting of dry soil as an indicator for biological soil quality, because they found that the CO_2 pulse was strongly correlated with soil microbial biomass and net N mineralization. We also observed a significant correlation between the flush of CO_2 after rewetting of air-dried soil and MBC under both aerobic and flooded conditions (Fig. 6.19). The pulse of CO_2 may be attributed not only to the killed microbial biomass but also to the increased availability of soil organic C due to increased exposure of organic surfaces during drying (Birch 1958; Orchard and Cook 1983, van Gestel et al. 1991). Hence, the flush of CO_2 was also found to be significantly correlated with HWEC but only under flooded condition (Fig. 6.19).

Since the flush of CO_2 after rewetting of dry soil would most probably come from the mineralization of the readily available C, the ratio of the initial flush of CO_2 to HWEC may also be used as an indicator of C-use efficiency or the extent of C losses during the drying and rewetting cycles in rice-wheat systems. Based on this ratio, a more efficient C use was exhibited by the residue-treated soils as compared with the control under aerobic incubation (Table 6.14).

The HWEC was significantly correlated with basal soil respiration under aerobic condition, and with PMN and MBC under both aerobic and flooded condition (Fig. 6.20) showing a strong influence of HWEC on soil biological activities and transformation

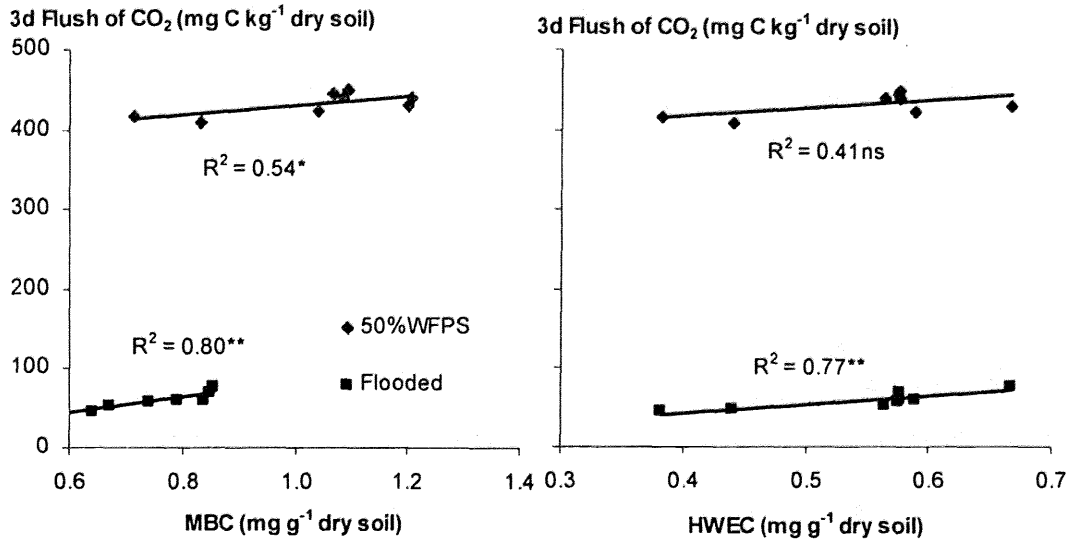


Fig. 6.19 Relationship of 3d flush of CO_2 with MBC and HWEC.

processes. HWEC was better correlated with PMN and MBC than MnOC (data not shown). There was a strong correlation between the increase in OC (decomposable fraction) in residue treated plots and HWEC (Fig. 6.21). According to Korschens (1997), the C content of the unfertilized plots represent the inert part of SOM and the decomposable part may be calculated from the difference in organic C of the plots with treatments of farm manure in combination with mineral fertilizers and the nil plots. Further, Schulz and Korschens (1998) also found a strong correlation between the decomposable part of SOM and HWEC. The strong correlations between HWEC and other biochemical characteristics indicate that it can be used as an integral measure of soil quality. Moreover, it is an easily measured parameter that can be used for monitoring short-term changes in the decomposable C pool as affected by soil management practices.

6.6.2 Long-term effects of organic and inorganic fertilization on soil parameters and soil quality in rice-wheat systems in India and Nepal

Yield decline with continuous rice-wheat cultivation in soils with various organic and inorganic amendments have been reported earlier from the 2 LTE in Ludhiana (Bhandari et al., 2002) and Bhairahawa (Regmi et al. 2002) but these have not been associated with changes in the SOM content since total soil organic C was maintained. However, this study has shown that soil HWEC or decomposable C was decreasing with the application of inorganic fertilizer alone (LT2) or in combination with GM (LT5) and WS (LT4) but not with FYM (LT3). An earlier report (Bhandari et al., 2002) showed a faster decline in

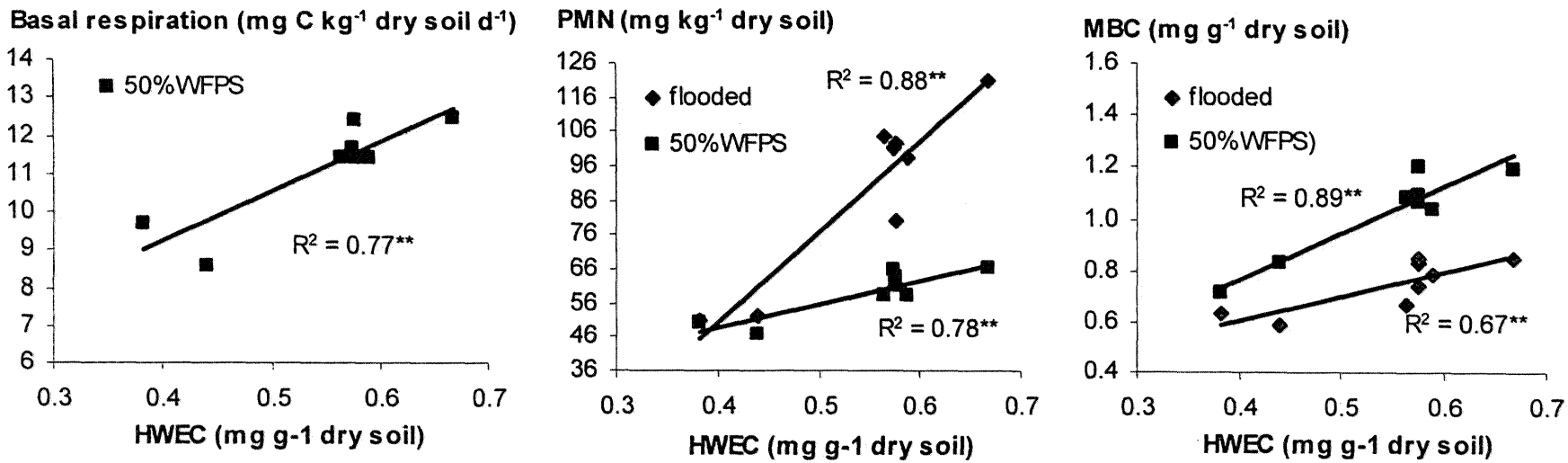


Fig. 6.20 Relationship of HWEC with basal soil respiration, PMN and MBC under aerobic and flooded condition.

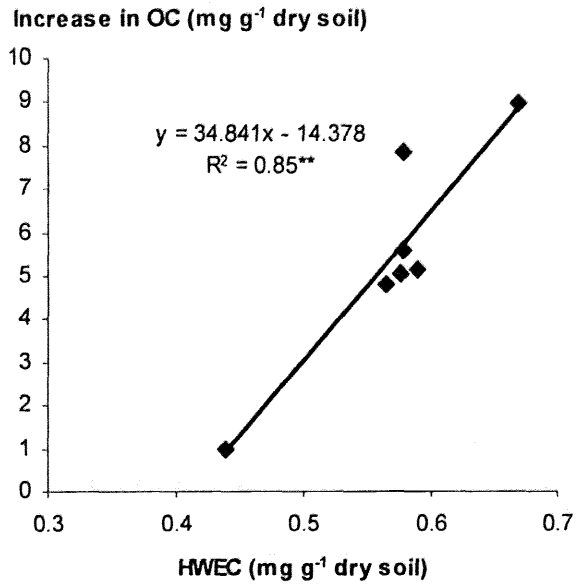


Fig. 6.21 Relationship between HWEC and the increase in OC with organic matter treatment.

yield in LT2 than in LT3. Moreover, an integrated analysis of yield trends in 19 LTE at various South Asian sites revealed a positive yield trend in the FYM+NPK treatment that was not observed with the NPK treatment alone (Chapter 5). Thus, rice grain yields across years in Ludhiana correlated with HWEC but not with total C (Fig. 6.22). Rice yields were lower with LT3 than with LT2q and with LT5 for the first 5 years and this may be

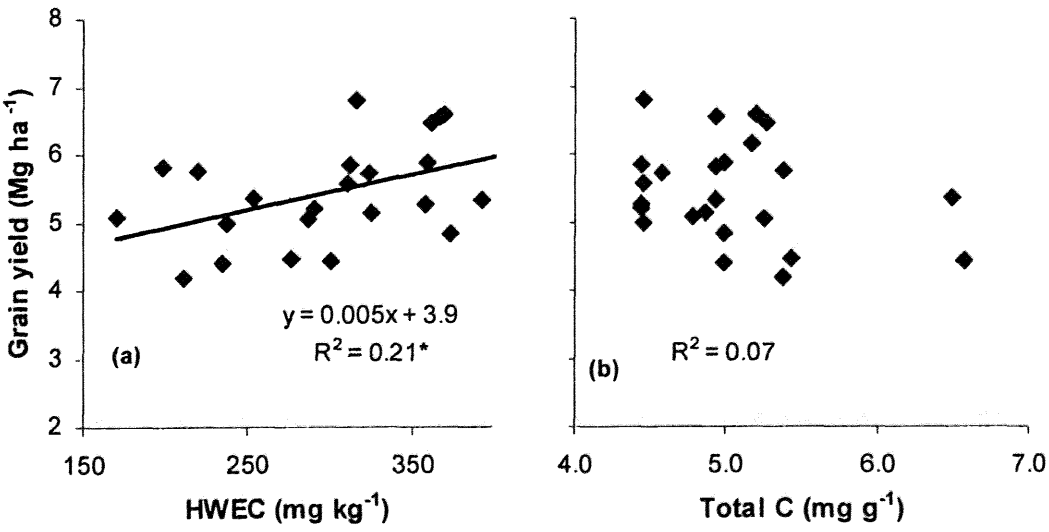


Fig. 6.22 Relationships of grain yield with HWEC and total C across years (Ludhiana, India LTE).

related to the faster N mineralization in GM than in FYM. From the 6th year, however, yields in LT2, LT3, and LT5 were not significantly different from each other (Bhandari et al., 2002) and only the treatment with FYM (LT3) showed a significantly higher total C, MnOC, HWEC, and total N in the soil than the unfertilized control during the 20th year. These observations indicate a greater impact of FYM in terms of C and N sequestration but not in terms of productivity.

In Bhairahawa, total and organic soil C in 2003 was highest in the FYM+NP treatment, following a trend similar to that of rice grain yield (Regmi et al., 2002). The HWEC and MnOC were not different among treatments in 2003, but rice grain yield correlated with both total C and HWEC across years in Bhairahawa (Fig. 6.23).

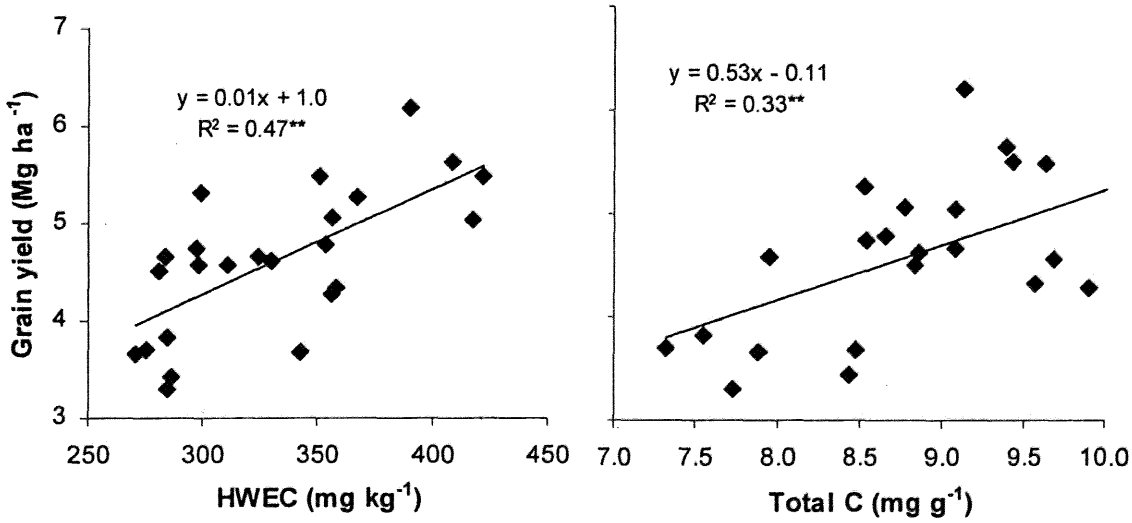


Fig. 6.23 Relationships of grain yield with HWEC and total C across years (Bhairahawa, Nepal LTE).

After 20 years, the soil HWEC in the NPK treatment in Ludhiana has fallen below 200 mg kg^{-1}dry soil (Fig. 6.12), the critical limit set by Körschens et al. (1998) for SOM depletion. However, with continuous application of FYM, the SOM level was maintained within the medium range (250-300 mg kg-1) for 20 years. The mean values of HWEC in the GM and WS treatments were also higher than the critical limit but were not significantly different from that of the NPK treatment. The decomposable SOM and its N release, and N+P supply from FYM combined with mineral fertilizer, are essential for crop growth and yield. Thus, FYM is crucial for maintaining soil fertility and enhancing

the sustainability of rice-wheat systems, especially in sandy soils with low organic matter content.

In Bhairahawa, the SOM level in all treatments after 15 years was within the medium to high (300-400 mg kg-1) range based on the values obtained for HWEC (>250 mg kg-1 dry soil). Consequently, rice grain yield was being maintained even in treatments with only inorganic fertilizer. In this LTE, P and not FYM has mitigated the rice yield decline. Regmi et al. (2002) have also suggested that the K in FYM mitigated the yield decline in wheat. However, a significant decline in HWEC was also observed over the years in Bhairahawa although there was no significant treatment x year interaction. Continuous monitoring of decomposable C with and without organic amendments in this LTE would provide valuable information for maintaining the soil fertility of rice-wheat systems in this area. Moreover, although there were no significant differences in HWEC among treatments after 15 years, total C and MnOC, which may represent the less labile C fraction, were significantly higher in the FYM treatment than in the control.

Aside from showing significant increases in total organic C and/or MnOC over the inorganically fertilized control, and preventing a decline in HWEC and total N, the FYM treatment significantly increased CEC, PMN and available P over the control at one or both of the LTE. The increase in MnOC in the FYM treatment (Fig. 6.13) in Ludhiana suggests an accumulation of lignin, which is being oxidized by $KMnO_4$. The decomposition of organic matter seems to slow down with time, resulting in a declining trend in HWEC and PMN but an increasing trend in MnOC.

The cumulative effects of organic amendments on MBC and soil respiration were not yet clearly manifested after 15 and 20 years of continuous cropping in Bhairahawa and Ludhiana. Nevertheless, an enhanced biological activity due to organic amendments based on increases in DA was observed in Ludhiana. The 40-yr rice-wheat LTE in Fukuoka, Japan, showed significant increases in MBC measured from incubated soils due to residue treatments unlike the 20- and 15-yr old LTE in Ludhiana and Bhairahawa. The amount of organic C incorporated as rice and wheat straw in the Fukuoka LTE was more than twice that of Ludhiana and Bhairhawa.

In Ludhiana, the accumulation of C and N from applied FYM was greater than that from GM and WS although the C:N ratio of WS was much higher than that of FYM, indicating that the ability of C and N to accumulate in soils does not depend only on the C:N ratio and quantity of organic matter but also on the quality of the organic matter applied. Further, the abilities of organic materials to supply nitrogen, increase soil fertility,

and accumulate in soils differ according to their origin and processing. With an FYM application rate of 200 Mg ha^{-1} yr^{-1}, which is about 40 times that in Ludhiana and Bhairahawa, a 35% accumulation of the C applied was estimated in European LTE (Körschens et al., 1998) vs. 11-23% C accumulation in Ludhiana and Bhairahawa. Further studies should be done at the farm level to improve the quality and optimize the rate of FYM and other organic materials applied to rice-wheat soils to minimize C and N losses to the atmosphere and groundwater. An optimal use of organic farm waste materials to supplement inorganic fertilizers could alleviate the yield decline and sustain the environment.

CHAPTER 7
GENERAL DISCUSSION AND CONCLUSIONS

7.1 General Discussion

Modern agriculture, though highly productive, is dependent on nonrenewable resources and is causing wide scale environmental contamination. Intensive cultivation of short duration high-yielding varieties (HYV), requiring large doses of N fertilizer, increased crop productivity. However, this proved to be detrimental to soil fertility, with the removal of large amounts of N from the system, and a depletion of organic matter due to the gradual replacement of organic manures as sources of plant nutrients. Additions of free N through biological N_2- fixation can balance the loss of N through crop harvest and other mechanisms (Ladha et al., 1997). From evaluations of indigenous algal biomass in rice fields and their N contents, it was estimated that the potential N contribution of a N_2-fixing bloom is around 13-25 kg N ha^{-1}. The low C:N ratio of BGA from field samples (average of 8.5) indicates that it has a better N availability than organic fertilizers such as FYM and GM. In this study, it was shown by ^{15}N-pot and field experiments that BGA-N was readily available to the rice crop with availability ranging from 27 to 36% for the first and second crops when BGA was incorporated in the soil and 14 to 23% when it was surface applied. An enhanced availability of BGA-N was observed by incorporation due to an accelerated decomposition of BGA in the anaerobic soil layer and a better proximity of the released nitrogen to the roots. This condition usually happens when an algal bloom develops later during the crop cycle, dries up on the soil surface, be incorporated by plowing, and start to decompose only at the beginning of the next growth cycle. Further, the pot experiment demonstrated that for the first crop, algal nitrogen was less available than ammonium sulfate but for two crops its availability was very similar to that of ammonium sulfate indicating the slow release nature of BGA-N.

Free N could also be derived from heterotrophic and rice-plant-associated N_2-fixation which was found to vary among rice genotypes. It is desirable to find rice varieties with the trait to stimulate N_2-fixation from among those with a higher harvest index or nitrogen harvest index. From acetylene reducing activity (ARA) measurements, rice-plant associated N_2-fixation was estimated at 1-5 kg N ha^{-1} $crop^{-1}$. Infrequent and insignificant inhibition of ARA at the early growth stages, and frequent and significant stimulation of ARA at the later stages due to different

fertilizers were found. Inorganic and organic fertilizers may exert different effects on N_2-fixation associated with rice. One of these could be the inhibitory effects due to the combined form of N (as in the case of inorganic fertilizer) or to toxic compounds released by the decomposition of organic materials. The former may be more important than the latter. Such inhibitory effects, however, may not be very important and long lasting, especially in tropical rice fields, where there is intense biological activity. Furthermore, the inorganic N disappears in flooded soil systems after 30-50 days of application of fertilizer (Watanabe and Inubushi, 1986; Nagarajah, 1987). The second type of effect may be a direct or indirect stimulatory effect. The direct effect could be due to the release of carbon substrates and other nutrients from the organic fertilizers, and the indirect effect could be increased plant growth and greater release of carbon compounds by the plant The significant increases in specific N_2-fixing activity and the total plant dry weight at heading stage in mungbean- and *Crotolaria*-incorporated treatments are interesting and noteworthy. It may be worthwhile to investigate whether the rhizobia, which are being inoculated in large numbers through the incorporation of its host biomass, are making an active N_2-fixing association with the rice plant. Rhizobia of some legumes such as Sesbania rostrata used as GM in rice has been reported to be fixing N_2 ex planta (Dreyfus et al, 1983).

The N contribution from heterotrophic and rice plant-associated N_2-fixation may be low, but in many farming systems, the effects of N_2-fixation and N fertilizer can be complementary in attaining high productivity. The integration of a legume crop in a cereal farming system is beneficial for the addition of fixed N, thereby enabling some reduction in the amount of fertilizer N required, as well as for reducing disease build-up. In the long-term, the global demand for N fertilizer may be reduced by improving the amount of N fixed and by increasing the integration of N_2-fixing species into farming systems.

The development and preferential planting of crops and crop varieties that have higher N uptake and/or utilization efficiency are essential, as it will result in lower N input requirements. Differences in GY among genotypes at suboptimal N levels may be attributed to variability in both N uptake and NUE. Efficient genotypes may be described as those which produce high grain yields at suboptimal N levels through increased N uptake and/or a more efficient utilization of the N taken up for grain production. A lower straw N concentration at maturity may indicate higher NUE. Straw N concentration of a genotype at maturity may not be significantly affected by

the change in available soil N. Grain N concentration also decreased by an average of 0.1% as NUE increase by 10. Hence the nutritional value of rice is not really sacrificed with the improvement of NUE. Genotypes with superior NUE and consistently good yield at suboptimal N levels have been identified. Further assessment of their yield performance and N uptake patterns at higher N levels and different sites is needed to test their stability and elucidate the mechanism for N uptake difference.

Monitoring long-term changes in crop yields is essential in evaluating sustainability of intensive agriculture and the efficacy of measures used to enhance productivity. In this paper, an integrated analyses of yields obtained from 50 LTE, using the random regression coefficient analysis (RRCA) and meta-analysis, indicated a significantly negative rice yield trend suggesting widespread yield decline in both rice-rice and rice-wheat systems of Asia. Unlike for rice-rice (RR), the system productivity of rice-wheat (RW) had no change. The stable yield trends in wheat contributed to the sustainability of the rice-wheat system although total crop productivity of rice-rice is about 15% higher than the rice-wheat system.

The integrated analysis of rice yield trends in RW systems across Asia also show that the addition of FYM affected the rice yield trend as shown by the significant year x treatment (NPK vs. NPK+FYM) interaction and the significant positive yield trend obtained in the FYM+NPK treatment which was not observed in the NPK treatment. However, the present results do not provide sufficient evidence on the increase in rice yield due to FYM in the long term (beyond 15 years) since most of the LTE ran for only 15 years at most. Only 2 LTE, which ran for more than 20 years have demonstrated this effect. Moreover, since most of the LTE ran for only 15 years we can only conclude that there was a positive linear trend in the FYM treatment up to 15 years at least. There is a need to analyze subsequent data to determine the trend beyond 15 years. Yields with FYM would tend to plateau at some point just as SOM formation and decomposition is expected to reach a "steady state" after a long period under the same soil management.

Soil organic matter (SOM), the key attribute of soil quality can be divided into labile or rapidly decomposed, and stable or slowly decomposed fractions. These fractions describe the quality of soil organic matter. Soil C oxidized by neutral $KMnO_4$, or permanganate-oxidizable C (MnOC), has been used as an index of labile C by several workers, although the nature of organic C oxidized has not been well

elucidated. However, results from this study indicate that MnOC is a better indicator of lignin content than labile C and thus it may be used to monitor changes in the stored organic matter or the slow C pool resulting from various agronomic practices. On the other hand hot water-extractable C (HWEC) was used to measure the readily decomposable C. Hot water-extractable C did not correlate with total C across years. This lack of correlation makes it important to monitor HWEC together with total C or MnOC in assessing the change in soil quality over the years. Studies on soil organic matter require that at least two fractions are considered, one being relatively inert, the other being decomposable and thus dependent on soil and crop management. Hot water extractable C was also found to correlate well with microbial biomass C (MBC) and potentially-mineralizable N (PMN) across years.

A comparison of soil properties between the unfertilized control and organic manure treatments after long periods (15-40yrs) in 3 LTE in Fukuoka, Japan; Ludhiana, India; and Bhairahawa, Nepal confirmed the long-term beneficial effects of organic manures on soil quality. The longest running LTE (40-yrs, in Japan) showed remarkable increases in the total, stable (MnOC) and decomposable (HWEC) soil C fractions, total N, available P and K, cation exchange capacity (CEC) and soil microbiological properties including MBC, potential mineralizable N (PMN), basal respiration, and 3-day flush of CO_2 following rewetting of dried soil, as affected by crop residue treatments (rice straw, composted rice straw, and rye grass/wheat straw) with or without inorganic N. In the Ludhiana- (20-yrs) and Bhairahawa-LTE (15-yrs) however, significant increases in total C, MnOC, CEC and Olsen P were observed with FYM treatment but the cumulative effects of organic amendments on microbiological properties - MBC and soil respiration were not clearly manifested. Nevertheless, an enhanced biological activity due to organic amendments based on increases in dehydrogenase activity (DA) was observed in Ludhiana. It should also be noted that the amount of organic C incorporated as rice and wheat straw in the Japan LTE was more than twice that of Ludhiana and Bhairhawa. Among the organic residue treatments in the Japan LTE, composted rice straw had the greatest impact in terms of improving or maintaining the soil quality and C and N sequestration. Greater C and N accumulation (Table 6.11) was observed from composted rice straw than from uncomposted rice or wheat straw indicating that composting plant residues before incorporation immobilizes C and N and slows down the C and N mineralization rate (Table 6.11).

In Ludhiana, trends of total C and MnOC over the years were stable with only NPK treatment, but was increasing with FYM treatment. On the other hand, decomposable C showed decreasing trends in all treatments except for the FYM treatment in Ludiana. After 20 years, the soil HWEC in the NPK treatment in Ludhiana has fallen below 200 mg kg^{-1}dry soil (Fig. 6.12), the critical limit set by Körschens et al. (1998) for SOM depletion. However, with continuous application of FYM, the SOM level was maintained within the medium range (250-300 mg kg-1) for 20 years. The mean values of HWEC in the GM and WS treatments were also higher than the critical limit but were not significantly different from that of the NPK treatment. The decomposable SOM and its N release, and N+P supply from FYM combined with mineral fertilizer, are essential for crop growth and yield. Thus, FYM is crucial for maintaining soil fertility and enhancing the sustainability of rice-wheat systems, especially in sandy soils with low organic matter content.

In Bhairahawa, the SOM level in all treatments after 15 years was within the medium to high (300-400 mg kg^{-1}) range based on the values obtained for HWEC (>250 mg kg-1 dry soil). Consequently, rice grain yield was being maintained even in treatments with only inorganic fertilizer. In this LTE, P and not FYM has mitigated the rice yield decline. Regmi et al. (2002) have also suggested that the K in FYM mitigated the yield decline in wheat. However, a significant decline in HWEC was also observed over the years in Bhairahawa although there was no significant treatment x year interaction. Continuous monitoring of decomposable C with and without organic amendments in this LTE would provide valuable information for maintaining the soil fertility of rice-wheat systems in this area. Moreover, although there were no significant differences in HWEC among treatments after 15 years, total C and MnOC, which may represent the less labile C fraction, were significantly higher in the FYM treatment than in the control.

Aside from showing significant increases in total organic C and/or MnOC over the inorganically fertilized control, and preventing a decline in HWEC and total N, the FYM treatment significantly increased CEC, PMN and available P over the control at one or both of the LTE in Ludhiana and Bhairahawa. The increase in MnOC in the FYM treatment in Ludhiana suggests an accumulation of lignin, which is being oxidized by $KMnO_4$. The decomposition of organic matter seems to slow down with time, resulting in a declining trend in HWEC and PMN but an increasing trend in MnOC.

In Ludhiana, the accumulation of C and N from applied FYM was greater than that from GM and WS although the C:N ratio of WS was much higher than that of FYM, indicating that the ability of C and N to accumulate in soils does not depend only on the C:N ratio and quantity of organic matter but also on the quality of the organic matter applied. Further, the abilities of organic materials to supply nitrogen, increase soil fertility, and accumulate in soils differ according to their origin and processing. With an FYM application rate of 200 Mg ha^{-1} yr^{-1}, which is about 40 times that in Ludhiana and Bhairahawa, a 35% accumulation of the C applied was estimated in European LTE (Körschens et al., 1998) vs. 11-23% C accumulation in Ludhiana and Bhairahawa. Further studies should be done at the farm level to improve the quality and optimize the rate of FYM and other organic materials applied to rice-wheat soils in order to minimize C and N losses to the atmosphere and groundwater. An optimal use of organic farm waste materials to supplement inorganic fertilizers could alleviate the yield decline and sustain the environment.

For best plant performance, it is essential to achieve an integrated N management that makes the best use of all available N sources both organic and inorganic. Agronomic and soil management practices involving the combined use of inorganic and organic fertilizers that also enhance biological nitrogen fixation would be ideal in improving or maintaining soil fertility, soil quality and productivity.

7.2 Conclusions:

1. A N_2-fixing algal bloom in the rice field contributes 13-25 kg N ha^{-1} while heterotrophic and rice plant-associated BNF contributes around 1-5 kg N ha^{-1} $crop^{-1}$. Nitrogen from BGA is released more slowly and is less susceptible to losses than that from ammonium sulfate.
2. Straw application enhanced heterotrophic and plant-associated N_2-fixation and increased the population of total and N_2-fixing heterotrophs in the soil. Higher bacterial population was observed with surface application of straw as compared with incorporated straw. Nitrogen fixation per plant (or per unit area) was either enhanced or not affected by the application of inorganic N and organic fertilizers. However, when expressed on a per unit plant dry weight basis, the ARA was generally lower except in cases where some legume green manures such as *V. radiata* and *Crotolaria* were incorporated.

3. Plant-associated BNF as measured by acetylene-reducing activity (ARA) differed among rice varieties and differences were most evident at heading stage.
4. Genotypes which may possess promising traits for improved N uptake and utilization efficiency were identified. High N uptake and NUE were observed in IR131429-150-3-2-1-2 (NUE 65.4, N uptake 9.1 g m-2) in the early-duration group, IR44 (NUE 67.2, N uptake 8.3 g m-2) in the medium duration group, and IR39323-182-2-3-3-2 (NUE 64.8, N uptake 9.3 g m-2) in the late-duration group.
5. An integrated analysis of rice yield data from rice-rice (RR) and rice-wheat (RW) long-term experiments (LTE) in Asia showed a declining trend with only inorganic fertilization and a positive trend when combined with farmyard manure (FYM). However, the initial yield was generally lower with FYM than without, that a yield increase due to FYM was observed only after 15 years.
6. Permanganate-oxidizable C (MnOC) is a better indicator of lignin content than labile C. Thus it may be used to monitor changes in the stored organic matter or the slow C pool resulting from various agronomic practices.
7. The current practice of inorganic fertilization alone can not maintain soil quality needed to sustain current levels of crop productivity. However, organic manures especially composted rice straw and FYM combined with inorganic fertilizers showed improvements in productivity and chemical and microbiological soil properties in the long-term.
8. MnOC but not hot-water-extractable C (HWEC) correlated with total C across years. MnOC increased over the years while HWEC decreased with the application of inorganic fertilizer alone or in combination with GM and WS but not with FYM. HWEC was found to correlate well with microbial biomass C (MBC), initial flush of CO_2 after rewetting dried soil, and potentially-mineralizable N (PMN), which also decreased across years, suggesting that HWEC has a strong influence on soil biological activities.

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