

**CARBON SEQUESTRATION AND GREENHOUSE GAS EMISSION
STUDIES IN SRI AND CONVENTIONAL SYSTEMS OF
RICE CULTIVATION**

By

S. K. RAJKISHORE

I.D. No. 09-809-001

**DEPARTMENT OF ENVIRONMENTAL SCIENCES
DIRECTORATE OF NATURAL RESOURCE MANAGEMENT
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE - 641 003**

2013

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Thesis submitted in part fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL SCIENCES
to the Tamil Nadu Agricultural University, Coimbatore-641 003

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CERTIFICATE

This is to certify that the thesis entitled “**CARBON SEQUESTRATION AND GREENHOUSE GAS EMISSION STUDIES IN SRI AND CONVENTIONAL SYSTEMS OF RICE CULTIVATION**” submitted in part fulfillment of the requirement for the degree of **DOCTOR OF PHILOSOPHY in ENVIRONMENTAL SCIENCES** to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research work carried out by **S. K. RAJKISHORE** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Place : Coimbatore

Date : 10/12/2012


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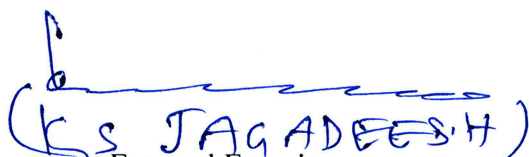
Members

:


(Dr. M. MAHESWARI) 23/13


(Dr. K.S. SUBRAMANIAN) 23/13


(Dr. P. GOVINDARAJU) 23/13


(K.S. JAGADEESH)
External Examiner
Date: 2-3-13

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- Sir Isaac Newton

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Now, I realize that Doctoral Program is just a small step in the journey to become a scientist. But the journey I travelled so far has already made me a theist.

*All the praise to God, for His Mercy has given me
patience and strength to complete this work.*


(RAJKISHORE)

Abstract

ABSTRACT

CARBON SEQUESTRATION AND GREENHOUSE GAS EMISSION STUDIES IN SRI AND CONVENTIONAL SYSTEMS OF RICE CULTIVATION

By

S.K. RAJKISHORE

Degree : **DOCTOR OF PHILOSOPHY (ENVIRONMENTAL SCIENCES)**

Chairman : **Prof. Dr. P. DORAISAMY**

Deputy Registrar (Administration)

Tamil Nadu Agricultural University

Coimbatore – 641 003

2013

Carbon sequestration pattern and greenhouse gas (GHG) emission potential of two distinct rice ecosystems (SRI and Conventional Systems) were studied through field and laboratory experiments at the Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore. For field experiments, treatments consisted of two systems of rice cultivation in main plot and four levels of nitrogen (0, 50, 100 and 150 kg ha⁻¹) replicated four times in a split plot design. The experiments were repeated in summer and *kharif* seasons of 2011. During the experimentation, carbon dynamics in SRI and conventional systems of rice cultivation besides methane emission pattern of these systems were evaluated.

The active (microbial biomass carbon and dissolved organic carbon) and passive (humic acid, fulvic acid, humin and glomalin) pools of soil organic carbon were determined during the critical growth stages namely active tillering, panicle initiation, flowering and harvest. The data showed that both active and passive pools of carbon were consistently and significantly higher in SRI than conventional system of rice cultivation with an exception of humin. However, the total carbon stock measured in terms of soil organic carbon was found to be higher in conventional system of rice cultivation. Similar responses were observed in both summer and *kharif* seasons. The incremental addition of nitrogen has progressively and linearly increased both active and passive pools of carbon regardless of growth stages or experimental seasons with an exception of soil organic carbon which decreased consistently.

Despite the fact that the rice plants in SRI were superior in terms of highest number of tillers, productive tillers and root biomass per hill, our observations on these parameters including the above ground and below ground biomasses per unit area basis revealed a different scenario. The conventional system recorded higher number of productive tillers and root biomass per m² than SRI. This suggests that the reduction in population as a consequence of wider spacing i.e., 25 cm x 25 cm to the tune of 68 per cent in comparison to conventional system is hardly compensated by the enhanced production of productive tillers per hill and biomass. This observation is very unique to bring out a critical attribute that contributes for the total crop productivity under conventional system in comparison to SRI. The biomass of roots also registered similar trend of response as that of shoot biomass. Both summer and *kharif* seasons have exhibited similar responses. The increasing levels of nitrogen have increased both shoot and root biomasses irrespective of growth stages or season.

In order to assess the unique advantage of SRI system of rice cultivation, the methane and carbon dioxide emissions were monitored during the experimental period especially during four times of cono-weeding. The results showed that SRI accounts for 19 to 63 percent reduction in methane emission in comparison to conventional system during *kharif* season as a consequence of cono-weeding. The cumulative methane emission for the entire cropping season has shown that SRI registered consistently lower values (31.8 and 37.7 kg ha⁻¹) than conventional system of rice cultivation (44.6 and 55.5 kg ha⁻¹) in summer and *kharif* seasons, respectively. Thus, there is a total reduction of methane emission by 29 and 32 per cent in SRI during summer and *kharif* seasons, respectively. Nitrogen fertilization had correspondingly increased the methane emission rates regardless of stages of observation or system of rice cultivation. Conversely, the cumulative CO₂ emissions in SRI (summer, 46 kg ha⁻¹; *kharif*, 88 kg ha⁻¹) were higher in comparison to conventional system of rice cultivation.

With a view to gain insights into the mechanisms involved in methane mitigation potentials of SRI, the biological activities especially methanogens and methanotrophs were enumerated periodically. The data on these microbial populations have shown that methanogens were found to decrease in SRI as a consequence of constant churning besides maintenance of alternate wetting and drying pattern of irrigation management. On the

other hand, the methanotrophs had increased in SRI due to the fact that this system facilitates aeration. Nitrogen fertilization has stimulated the activities of methanogens while inhibiting the activities of methanotrophs. Such paradigm shift in microbial diversity was evident during the entire course of experimentation.

The response of rice plants to elevated CO₂ concentrations were studied in open top chamber (OTC) conditions. The treatments consisted of three levels of CO₂ (380, 550 and 750 µmol mol⁻¹) and three levels of nitrogen (0, 150 and 200 kg ha⁻¹) and replicated five times in completely randomized design. During the experimentation, the carbon sequestration pattern and nitrogen assimilation in rice plants were examined. The data showed that elevated levels of CO₂ had least impact on soil organic carbon while accelerating active pools of carbon comprising microbial biomass carbon and dissolved organic carbon. The carbon accumulation in above ground biomass at the harvest stage tended to be increased with elevated levels of CO₂. The key enzymes involved in N assimilatory pathway namely nitrate reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase were registered higher at the early stage of crop growth (tillering) and decreased with the advancement of crop growth. N fertilization had positive effects on N assimilatory enzymes. Further, the methane emission rates were monitored regularly during the experiment period. The results revealed that elevated [CO₂] had increased the methane emissions regardless of stages of crop growth. Interestingly, it is observed that elevated [CO₂] enhanced the grain productivity but affected the quality of rice grains. The existing recommended dose of N in combination with elevated CO₂ levels has deleterious effect in producing rice grains with deficient levels of nitrogen.

Overall, the data on carbon sequestration pattern of two distinct systems of rice cultivation have indicated that SRI had a unique advantage of conserving both passive and active pools of carbon besides minimizing methane emission. Despite the fact that SRI could be one of the potential methane mitigation strategies, the productivity per unit area was observed to be lower than conventional system of rice cultivation. This necessitates modifying the spatial geometry of SRI and manuring practices to gain the twin advantage of enhanced productivity while circumventing methane emission from rice fields. The experiments on elevated CO₂ concentrations have clearly demonstrated that CO₂

enrichment will favourably improve the productivity and nutritional quality only when the rice plants are nourished with excessive amounts of nitrogen. These experiments have opened up new set of avenues to look at the unique features of SRI closely to maximize the benefits of environmental safety without compromising crop productivity.

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Abbreviations

ABBREVIATIONS

[CO ₂]	:	Carbon di oxide concentration
CD	:	Critical difference
CFU	:	Colony Forming Units
DAT	:	Days After Transplanting
DOC	:	Dissolved Organic Carbon
EC	:	Electrical Conductivity
<i>et al</i>	:	Co-workers
FA	:	Fulvic acid
Fig	:	Figure
GDH	:	Glutamate Dehydrogenase
GHG	:	Greenhouse Gas
GOGAT	:	Glutamate Synthase
GS	:	Glutamine Synthetase
HA	:	Humic acid
IPCC	:	Intergovernmental Panel on Climate Change
MBC	:	Microbial Biomass Carbon
MBN	:	Microbial Biomass Nitrogen
MMO	:	Methane monooxygenase
N	:	Nitrogen
NH ₄ ⁺	:	Ammonium
NO ₃ ⁻	:	Nitrate
NRase	:	Nitrate reductase
NS	:	Not Significant
OTC	:	Open Top Chamber

$P \leq 0.05$	:	Probability at 5% level of significance
$P \leq 0.01$	:	Probability at 1% level of significance
ROS	:	Reactive Oxygen Species
SEd	:	Standard error difference
SOC	:	Soil Organic Carbon
SOD	:	Super oxide dismutase
SOM	:	Soil Organic Matter
SRI	:	System of Rice Intensification
TPF	:	Triphenyl formazon
<i>viz</i>	:	namely

UNITS

% of OM	:	per cent of organic matter
%	:	per cent
μg	:	microgram
μM	:	micro molar
dS m^{-1}	:	deci Siemens per meter
g pot^{-1}	:	gram per pot
kg ha^{-1}	:	kilogram per hectare
mg kg^{-1}	:	milligram per kilogram
mg L^{-1}	:	milligram per liter
Mg m^{-3}	:	Megagram per cubic meter
mM	:	milli molar
Pg	:	Petagram (1 Pg = 1×10^{15} g)
tillers hill ⁻¹	:	tillers per hill

Introduction

CHAPTER 1

INTRODUCTION

Global warming is a serious matter of concern for people on earth in general and environmental scientists in particular. This matter came to limelight only in the year 1992 when the Earth Summit held in Rio de Janeiro brought to the notice of global community that earth has warmed up in the past century, which is more intense in the past few decades than the early part of the 20th century. The global warming event is closely related to the rise in greenhouse gas (GHGs) emissions such as CO₂, CH₄ and N₂O that contribute to the tune of 60, 20 and 6 per cent (IPCC, 1996). Despite several gases responsible for enhanced greenhouse effect, CO₂ emission is considered as a ‘kingpin’. It has been observed that the atmospheric CO₂ concentration was 280 ppm in the year 1850 (Etheridge *et al.*, 1996) and increased to 390 ppm in the year 2011 (Tans and Keeling, 2011) that commensurate with mean temperature rise of 0.8°C during the 20th century (IPCC, 2007). This trend continues to increase at an alarming rate which may result in serious consequences. The issue was discussed length and breadth in two major events associated with climate change namely, Third session of Conference of Parties (COP) at Kyoto in Japan (1997) and Copenhagen Summit (COP 15) in Denmark (2009) and the international communities were trying to evolve consensus to mitigate greenhouse gas emissions so as to prevent global warming impacts on the Earth planet. The industrially developed nations in the western world particularly US which is known to emit the highest per capita CO₂ (IEA, 2011), was not prepared to sign the Kyoto protocol agreement due to the fact that their GHGs emissions could not be minimized by 5.2 per cent of the 1990 emission level within the stipulated time frame of 2008 - 2012 (UNFCCC, 2011). On the other hand, in the eastern part of the world, the developing nation mainly China is reportedly the single largest emitter of CO₂ in the globe (IEA, 2011). In addition, India and other South East Asian countries are also often blamed for their intense GHGs emissions primarily from agriculture sector with the specific reference to rice cultivation.

Agriculture is critical for human survival and its importance for the environment and climate is less recognized. The global concern of the 21st century is food security to meet the demand of burgeoning population within the degrading natural resources.

Farming is further stressed by the impact of climate change. Nevertheless, there are apprehensions about the contribution of agriculture sector to greenhouse gas emissions. These challenges necessitate the development of agricultural practices that are environmentally friendly and resilient to climate change.

Rice continues to be a major food crop and serves as a staple diet for more than 700 million people in India (FAO, 2011). Despite the fact that the crop has been researched in various spheres focusing on productivity, the average per hectare yield hardly exceeds 5 tonnes. The area under rice cultivation is nearly 44 million hectares in the country, primarily under submerged system of cultivation. The submerged rice cultivation system is known to produce more grain yield in comparison to aerobic or semidry conditions. In this system of cultivation, the soil is kept at anaerobic condition that facilitates methane emission which has a global warming potential of 25 times as that of CO₂ for 100 yr time horizon (IPCC, 2006).

As efforts to mitigate climate change increase, there is a need to identify cost-effective ways to avoid emissions of GHGs. Agriculture is rightly recognized as a source of considerable emissions with concomitant opportunities for mitigation. Among the various mitigation options, the carbon sequestration in agricultural system is identified as a promising area by IPCC (Nakicenovic *et al.*, 2000) and it is often termed as a win-win or no-regret strategy owing to its numerous ancillary benefits such as improved soil quality and increased crop yield (Lal, 2008). Carbon sequestration can be defined as the transfer and net removal of atmospheric CO₂ into long-lived carbon pools such as biotic and pedologic by photosynthetic pathway of plants in association with microorganisms. In agro-ecosystems, soils have immense potential for carbon sequestration with the fact that every kilogram of soil organic carbon (SOC) represents 3.7 kilograms of CO₂ removed from the atmosphere (McConkey *et al.*, 1999). The potential for soil carbon sequestration in cropland soils ranges from 21 to 42 Pg, which can be achieved in a span of 50 years (Lal, 2001). The total soil carbon sequestration potential of Indian soils has been estimated as 39.3 to 49.3 Tg C y⁻¹ (Lal, 2004b) suggesting the significance of proper management of agricultural system to ensure environmental safety.

Rice soils are known to retain higher amounts of resilient carbon among all terrestrial ecosystems (Liu *et al.*, 2006; Stern *et al.*, 2007; Xie *et al.*, 2007). It is important to note that organic matter preferentially accumulates in continuous rice systems as a result of submerged conditions. Slower decomposition of organic matter and higher net productivity of submerged paddy soils lead to net carbon accumulation (Sahrawat, 2005). On the other hand, anaerobic conditions in this system of rice cultivation provides conducive environment for methanogens to produce methane. The annual methane emission from Indian rice fields is reported as 3.6 Tg per annum. This situation warrants the scientists to identify the eco-friendly system of rice cultivation to promote carbon sequestration *vis-à-vis* lessen the global warming impacts.

In the past few decades, rice scientists across the globe have taken painstaking efforts and developed conventional best management practices for rice farming. Though conventional system of rice cultivation has its own advantages, alternate systems of rice cultivation are mushrooming in order to augment rice productivity besides improving the input and water use efficiency. In the recent years, System of Rice Intensification (SRI) method of cultivation has received wide attention and it has even become a buzzword for many rice growers. Indeed, Tamil Nadu Agricultural University took a pioneering effort in the year 2000 to evaluate the efficiency of SRI by conducting first official field trials in the country. By 2008-2009, the Tamil Nadu State Government reported 7,50,000 ha under this new management (Uphoff and Mishra, 2009).

Though the significance of SRI is unequivocally demonstrated, no research attempts have been made to determine carbon sequestration pattern in SRI. The favourable factors that facilitate C sequestration in SRI include cono-weeding, profusely branched fibrous root systems, intense rhizosphere microbial activities, intermittent wetting and drying of soil, larger above ground and below ground biomasses besides enriching passive carbon pool. The proposed research program focuses on assessing the carbon sequestration besides estimating the methane emission in both SRI and Conventional System of rice cultivation which will serve as a data base to favour eco-friendly rice cultivation and promote better livelihood for rice farmers of major rice growing tracts.

The carbon sequestration pattern of rice eco-system is likely to be altered or modified under the various scenarios of climate change. Such events are often predicted by enriching the atmosphere with CO₂ under Open Top Chamber (OTC) conditions. It has also been estimated that the year 2050 and 2100 are the critical time frame for evaluation where the atmospheric CO₂ concentrations is likely to exceed 550 and 750 ppm, respectively. In order to assess the impacts of elevated CO₂ levels on carbon sequestration of rice ecosystem, this research program included an OTC experiment.

It is well known that rice plant possesses C₃ photosynthetic pathway and is less efficient due to the fact that the assimilated carbon get decarboxylated under the current atmospheric carbon dioxide concentrations. Thus, the elevated CO₂ is considered as carbon fertilization to promote photosynthetic activities and improving the productivity of crops. However, the other factors such as N fertilization, temperature and water may cause inhibitory or supplementary effects on carbon assimilation in rice plants. It is well documented beyond doubt that carbon and nitrogen cycles co-exist and complement each other (Reich *et al.*, 2006). Any alterations in one factor will up-regulate or down-regulate the biochemical pathways, but the mechanisms underlying such processes are poorly understood and need systematic investigations. In order to address these issues, OTC experiments were conducted by imposing elevated levels of carbon dioxide to the rice plants fertilized with varying doses of nitrogen.

This study hypothesized that System of Rice Intensification (SRI) which encompasses production of fibrous profusely branched root system, larger biomass productivity and exclusion of submergence that prevents methane emission will result in accumulation of active (biomass carbon) and passive (humic acid, fulvic acid, humin, glomalin) pools of C and facilitates in carbon sequestration. Such long lived C pools will contribute for the improved soil organic status and in turn helps in sustaining farm productivity without associated environmental harm.

In addition, the pot culture study hypothesized that availability and assimilation of nitrogen is a critical factor to rice plants exposed to elevated levels of CO₂ for harnessing the benefits of CO₂ fertilization effect. Hence, the present investigation focuses on assessing the carbon sequestration pattern in two distinct systems of rice cultivation

besides evaluating the physiological response of rice plants exposed to elevated atmospheric CO₂ concentrations under field and OTC experiments, respectively with the following objectives:

- To determine the seasonal changes in carbon sequestration in two distinct rice ecosystems (SRI and Conventional Systems).
- To assess the carbon flow in above ground and below ground biomasses.
- To estimate the temporal and seasonal changes in methane flux of rice ecosystems.
- To determine carbon sequestration pattern of rice under elevated CO₂ concentrations and nitrogen fertilization.

Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

This thesis focuses on assessing the carbon sequestration pattern in conventional and SRI system of rice cultivation. The literature review encompasses global carbon cycle, abiotic and biotic carbon sequestration, carbon dynamics in rice soils, pattern of carbon partitioning in rice ecosystem, methane mitigation strategies besides response of rice to elevated CO₂ concentrations. The literature review made in this chapter serves as a solid background to undertake the research in the emerging area of carbon sequestration in rice ecosystem.

2.1. Concerns of Enhanced Greenhouse Effect

Climate change is one of the most pressing modern-day environmental issues. Natural shifts in global temperatures have occurred throughout human history. However, the 20th century recorded a rapid increase in global mean surface temperature by 0.8°C as a result of increased greenhouse gas emissions (GHGs) through various anthropogenic sources (IPCC, 2007). This phenomenon of increased global mean temperature is termed as „enhanced greenhouse effect’. Carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are important drivers of this anthropogenic greenhouse effect. The atmospheric CO₂ concentration has increased steadily from 280 ppmv in the pre-industrial era (IPCC, 1995) to 390.5 ppmv in the year 2011 (Tans and Keeling, 2011). The importance of atmospheric concentration of CO₂ on global temperature was recognized by Arrhenius (1896) towards the end of the nineteenth century. Well documented scientific evidence suggests that among the GHGs, CO₂ accounts for 60 per cent of global warming (IPCC, 2001). Most climatologists agree that the warming trend will continue and at an accelerating pace unless necessary mitigation measures are addressed immediately.

2.2. Mitigation Strategies

There is a strong interest in stabilizing the atmospheric abundance of CO₂ and other GHGs to mitigate the unforeseen risks (Kerr, 2007; Walsh, 2007). There are three strategies of lowering CO₂ emissions to mitigate climate change (Schrag, 2007): (i) reducing

the global energy use, (ii) developing low or no-carbon fuel and (iii) sequestering CO₂ from point sources or atmosphere through natural and engineering techniques.

There are five global C pools (Fig. 2.1) namely, oceanic (38,000 Pg), geologic (5000 Pg), pedologic (2300 Pg to 1 m deep), atmospheric (760 Pg) and biotic (550 Pg) components (Lal, 2001). According to IPCC (1995), the enrichment of the atmospheric C pool has occurred at the expense of the geologic pool (fossil-fuel emissions estimated at $6.1 \pm 0.5 \text{ Pg C yr}^{-1}$), the biotic pool (tropical deforestation and wildfire, estimated at about $1.5 \pm 1.0 \text{ Pg C yr}^{-1}$) and the soil pool (cultivation and other anthropogenic disturbances). Hence, developing technologies to reduce the atmospheric concentration of carbon dioxide (CO₂) is an important issue to address.

a. Carbon sequestration

Carbon sequestration is defined as the transfer of atmospheric CO₂ into other long-lived global pools including oceanic, pedologic, biotic and geological strata to reduce the net rate of increase in atmospheric CO₂ (Lal, 2008). In other terms, it is the process of transfer and secure storage of atmospheric CO₂ into other long lived C pools. Therefore, the objective of carbon sequestration process is to balance the global C budget such that future economic growth is based on a „C neutral’ strategy of no net gain in atmospheric C pool. These options can be grouped into two broad categories namely abiotic and biotic sequestration.

(i) Abiotic carbon sequestration

This strategy is based on physical and chemical reactions including engineering techniques without intervention of living organisms. Abiotic carbon sequestration in oceanic and geological structures has received considerable attention (Freund and Ormerod, 1997) as it carries larger sink capacity than biotic sequestration. However, the principal concerns about abiotic sequestration is the reliability of storage of vast quantities of CO₂ and costly due to its slow rate of conversion besides energy expensive (Kintisch, 2007; Schrag, 2007).

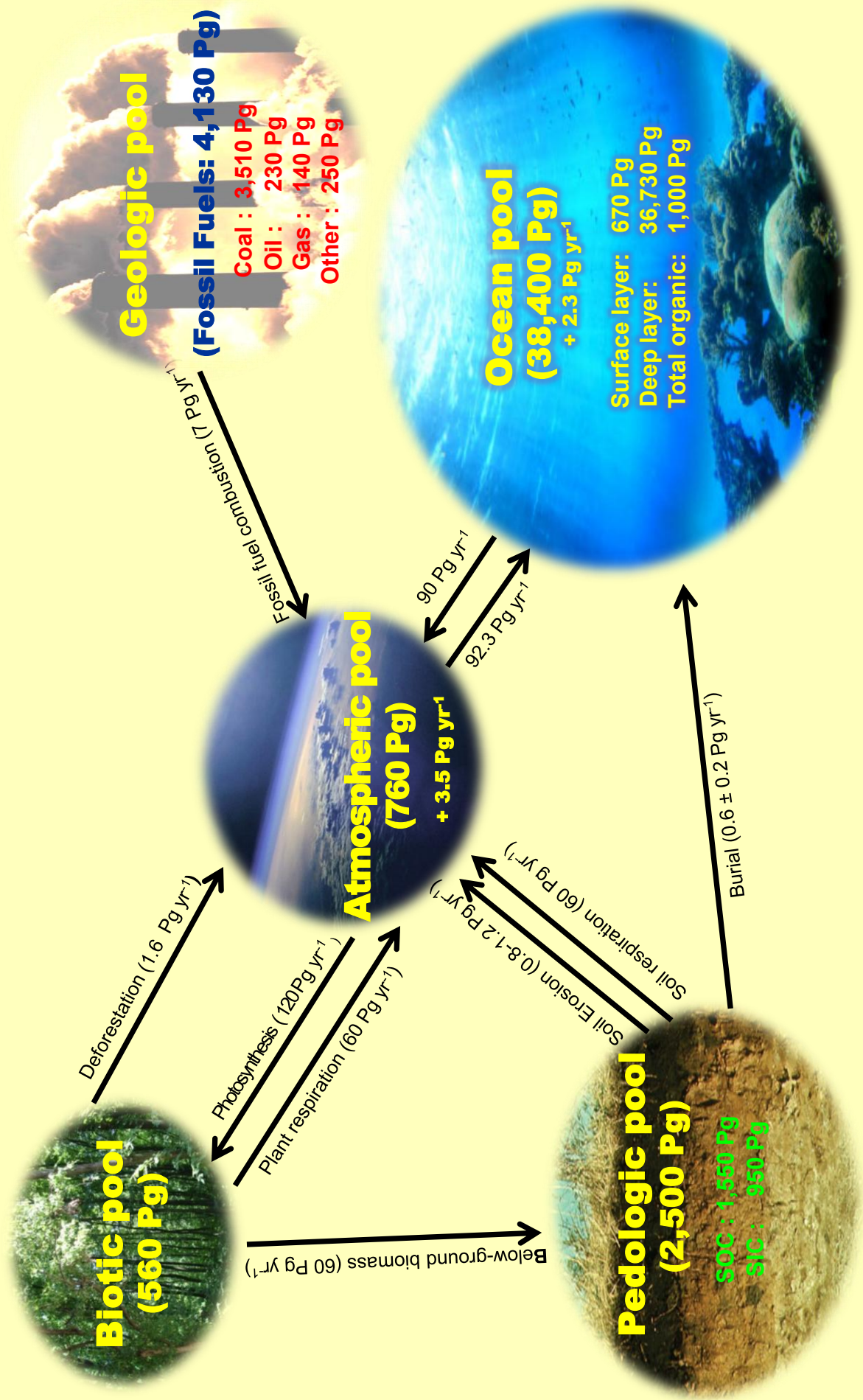


Fig. 2.1. Global carbon pools (Modified from Lal, 2008)

(ii) Biotic carbon sequestration

It is based on managed intervention of higher plants and micro-organisms in removing CO₂ from the atmosphere (Lal, 2008). The biotic carbon sequestration is broadly classified into oceanic and terrestrial sequestration. There are several biological processes leading to carbon sequestration in the ocean through photosynthesis. Phytoplankton's photosynthesis is one such mechanism which fixes approximately 45 Pg C yr⁻¹ (Falkowski *et al.*, 2000). However, with the current state of knowledge, the topic of ocean sequestration remains a debatable issue (Johnson *et al.*, 2002).

Terrestrial carbon sequestration refers to the transfer of atmospheric CO₂ into biotic and pedologic C pools. Of the 8.6 Pg C yr⁻¹ emitted into the atmosphere, only 3.5 Pg (or) 40 per cent of the anthropogenically emitted CO₂ remains in the atmosphere primarily owing to unspecified terrestrial sinks which sequesters atmospheric CO₂ and play an important role in the global C cycle (Lal, 2008). Terrestrial ecosystems constitute a major C sink owing to the photosynthesis and storage of CO₂ in live and dead organic matter. The terrestrial sink absorbs approximately 2–4 Pg C yr⁻¹ and its capacity may increase to approximately 5 Pg C yr⁻¹ by 2050 (Cramer, 2001; Scholes and Noble, 2001).

Three principal components of terrestrial C sequestration are soils, forests and wetlands. Forest ecosystems store carbon as lignin and other relatively resistant polymeric C compounds. Wetlands and the associated soils or histosols constitute a large pedologic pool estimated at approximately 450 Pg (Gorham, 1991). Wetland soils may contain as much as 200 times more C than the associated vegetation (Garnett *et al.*, 2001). For terrestrial ecosystems, it has been proposed that C sequestration can be increased by increasing soil C stocks (Batjes, 1998; Izaurralde *et al.*, 2001; Scholes and Noble, 2001). Such a proposal is sensible given the fact that more than 80 per cent of the terrestrial organic C stores are contained in soils (IPCC, 2000).

Owing to its numerous ancillary benefits (e.g. improved soil and water quality, restoration of degraded ecosystems, increased crop yield), terrestrial C sequestration is often termed as win–win or no-regrets strategy (Lal *et al.*, 2003).

2.2.1. Soil carbon sequestration

The role of world agricultural soils as a potential sink as well as source and its contribution cannot be overruled. Every pound of SOC represents 3.7 pounds of CO₂ removed from the atmosphere (McConkey *et al.*, 1999). Thus, soil carbon sequestration may be of great importance for mitigating the increase in atmospheric CO₂ concentration (Amundson, 2001). Global soil carbon (C) is 3.3 times the size of the atmospheric pool and 4.5 times the size of the biotic pool (Lal, 2004a). The carbon sequestration potential of agricultural soils was duly recognized in article 3.4 of the Kyoto Protocol and it is now considered as a possible means of reducing atmospheric CO₂ (Ramesh *et al.*, 2007).

Watson *et al.* (1996) estimated that 0.4–0.8 Pg C y⁻¹ is sequestered in agricultural soils globally by implementation of appropriate management practices. Numerous studies have shown that cropland soils may serve as a large sink for atmospheric CO₂ by enhancing SOC (Ogle *et al.*, 2005; Follett *et al.*, 2005).

In general, the soil carbon sequestration implies enhancing the concentration/ pools of soil organic carbon (SOC) and soil inorganic carbon (SIC) as secondary carbonates (Lal, 2008). However, the role of SIC in carbon sequestration is less well understood than that of SOC (Lal, 2001) and there are also concerns of degradation of fertile agricultural soils as a result of enhancing the soil inorganic carbon. Hence, research in the field of soil carbon sequestration currently focuses on improving the SOC content of agricultural soils, which can improve soil quality and crop yield while improving the environment (Lal, 2008).

With respect to soil carbon sequestration, it is most desirable to fix atmospheric C (upon photosynthesis) in those pools having long turnover times (Batjes, 2001). The SOC pools can be broadly classified into active and passive pools.

a. Active carbon pools

The SOC is comprised of labile or actively cycling pool and stable, resistant or recalcitrant pools with varying residence time. Parton *et al.* (1987) observed that active pools of SOC consist of living microbes and microbial products along with soil organic carbon with a short turnover time (1 to 5 yrs). Active carbon fractions are energy source

for the soil food web and thus influence in nutrient cycling (Chan *et al.*, 2001). Moreover, the labile fractions of SOC respond rapidly to changes in C supply and are considered to be important indicators of soil quality (Majumder *et al.*, 2008).

The major fractions of active pools of SOC include soil microbial biomass carbon, water soluble organic carbon and water soluble carbohydrates (Vivek, 2008). Carbohydrates represent approximately 5 to 20 per cent of the total soil organic carbon and originate from plants, animals and microorganisms (Stevenson, 1994). Soil carbohydrates are composed of a mixture of complex polysaccharides, which are composed of monosaccharides. Soil carbohydrates contribute to the formation and stabilization of soil structure and have been primarily studied because of their relation to soil aggregation.

The soil microbial biomass normally constitutes about 1–5 per cent of the total soil carbon and can provide an early warning for a possible degrading and/or aggrading effect of different management practices on soil quality (Mandal, 2005). The C:N ratio of microbial biomass carbon is around 5 to 15 and provides mineral nutrients and life to the soil (Swarup and Singh, 2009). Water soluble organic carbon seldom exceeds 200 mg kg⁻¹ but it appears to be the immediate organic substrate for soil microorganisms. Turnover of soil microbial biomass therefore requires replenishment of WSOC supplies (Vivek, 2008).

b. Passive carbon pools

Passive pools of SOC include the most recalcitrant fraction of soil organic matter. The fractions that are chemically recalcitrant are humic acid (HA) and fulvic acid (FA) with the longest turnover time (Singh, 2004). These humic substances are relatively stable fraction of SOM pool and are able to partly resist microbial decomposition and hence, they help to maintain the SOM level. At the same time, they possess a considerable chemical reactivity through which they contribute to the properties and productivity of soils (Stevenson, 1994). Highly recalcitrant or passive pools are very slowly altered by microbial activities (Sherrod *et al.*, 2005) and have the C:N ratio of 7:1 to 9:1 (Swarup and Singh, 2009). Thus humification (the alteration of biologically derived carbon to chemically complex forms) also represents a critical process driving carbon sequestration.

Glomalin, a recalcitrant mycorrhiza specific glycoprotein produced by symbiotic arbuscular mycorrhizal fungi substantially contributes for soil carbon sequestration besides facilitating soil fertility (Vivek, 2008; Subramanian *et al.*, 2009). Glomalin is a

component of the hyphal wall that accumulates in soils (Wright and Upadhyaya, 1996) where it is considered to have a slow turnover as a consequence of its long resilient time which varies from 50 to 500 years. The total contribution of glomalin C to total organic C pools was estimated to be from 3.77 to 7.84 per cent of total C depending on land-use type and total soil C (Rillig *et al.*, 2003). In addition, it contains iron up to 5 per cent and enhances soil aggregation (Wright and Upadhyaya, 1998 and Rillig *et al.*, 2001), thereby protecting carbonaceous material from rapid degradation in soils (Rillig *et al.*, 1999).

2.2.1.1. Effect of nitrogen on soil carbon sequestration

As one of the most important and widespread management practices, fertilizer application shows great impact on SOC storage and cycling (Kaur *et al.*, 2008 and Triberti *et al.*, 2008). Firstly, fertilization affects organic matter input to soil directly through organic amendments and/or indirectly by altering crop growth and residue return (Min *et al.*, 2003). Secondly, fertilizer application greatly influences soil aggregation (Tisdall and Oades, 1982), which exerts physical protection for SOC, thus controlling its decomposition and transformation (Jastrow *et al.*, 2007).

Halvorson *et al.* (1999) observed that fertilizer N rates of 67 and 134 kg N ha⁻¹ yr⁻¹ resulted in 140 and 182 kg ha⁻¹ yr⁻¹ more SOC, respectively in the 0-15 cm depth than for zero N treatment. In a further analysis, Halvorson *et al.* (2000) considered three additional long-term cropping systems studies and concluded that managing N fertilization for optimum yield significantly increased crop residue inputs at all locations and resulted in increased SOC levels. They demonstrated that the carbon sequestration efficiency was improved by N fertilization and contributed for improvement of soil quality.

In contrast, addition of FYM enhanced the content of SOC, whereas application of nitrogen fertilizer showed no effect (Su *et al.*, 2006; Yan *et al.*, 2007). Data from the World's oldest experimental site (Morrow Plots) under continuous corn (*Zea mays* L.) revealed that a net decline occurred in soil carbon despite massive residue C incorporation in the soil (Khan *et al.*, 2007) and the SOC build up was observed only when fertilization was practiced. These findings implicate that N fertilizers promote the decomposition of crop residues and soil organic matter although crop yields increased with nitrogen additions.

2.2.2. Carbon sequestration potential of rice soils

Rice cultivation globally covers a total area of about 153 million ha and it has been proposed to have a great potential in sequestering atmospheric CO₂ (Lal, 2004a and IPCC, 2007). Managed wetlands, in particular rice paddies, represent a promising distributed carbon. Wetlands play an important role in carbon cycling and among all terrestrial ecosystems they have the highest carbon density (Stern *et al.*, 2007). The high productivity, high water table, and low decomposition rate associated with wetlands lead to carbon storage within the soil, sediment and detritus (Whitting and Chanton, 2001).

Great potential for soil C sequestration has been found in rice paddies (Pan *et al.*, 2003 and Liu *et al.*, 2006). Jarecki and Lal (2003) evaluated the potentials for SOC sequestration for rice and concluded that 401 kg C ha⁻¹ yr⁻¹ with an average rice yield of 3.96 t ha⁻¹ and a C input in terms of crop residues of 2.67 t ha⁻¹ yr⁻¹. Evidence has also shown that carbon density in paddy soils was higher than that in upland soils (Xie *et al.*, 2007) and significant amount of carbon is sequestered in paddy soils over the last two decades in China (Huang and Sun, 2006).

Ramesh *et al.* (2007) investigated various land use systems of semi-arid tropics (SAT). They have shown that paddy systems had highest content of organic carbon and nitrogen irrespective of bioclimatic zones, land use under agricultural and horticultural systems.

Wu (2010) investigated the carbon sequestration potential of four landscape units namely lowland, low-hill, high-hill and karst-mountain. The mean values for SOC content in paddy soils (0 - 20cm) in the landscape units varied from 16.0 to 27.7 g kg⁻¹, which were higher in comparison to arable, orchard management and woodlands soils except in the karst-mountain. They concluded that paddy ecosystems in subtropical China had the ability to sequester organic C in amounts larger than those in other ecosystems.

2.2.2.2. Mechanisms of soil carbon sequestration in rice system

Increasing evidence is showing a greater potential for carbon (C) sequestration in paddy soils than in upland soils. However, the mechanisms underlying long-term accumulation and protection of soil organic carbon (SOC) in paddy fields have not been well documented (Huang *et al.*, 2010).

The classical literature by Ponnamperna (1972) explains that the decomposition of organic matter in a submerged soil differs from that in a well drained soil in two respects: it is slower and the end products are different. In a well drained soil, decomposition of plant residues is accomplished by a large group of microorganisms assisted by the soil fauna. Owing to the high energy release associated with the aerobic respiration of these organisms, decomposition of substrate and synthesis of cell substance proceed rapidly.

Under anaerobic conditions, both the decomposition of amended organic matter and mineralization of native SOC are lower than those under aerobic conditions (Witt *et al.*, 2000; Guo and Lin, 2001). Intensive rice cropping has led to accumulation of phenolic lignin compounds that are resistant to microbial decomposition under submergence (Olk *et al.*, 2000). They concluded that the changes in SOC quality under anaerobic conditions may contribute to the slowdown of C decomposition in paddy soils.

The mechanisms involved in preferential accumulation of organic matter in wetland soils are ascribed mainly to anaerobiosis and the associated chemical and biochemical changes that take place in submerged soils (Sahrawat, 2004b). In submerged soils, the formation of recalcitrant complexes with organic matter renders them less available for microbial attack. Moreover, the biological nitrogen fixation coupled with overall higher primary productivity and decreased humification lead to net accumulation of organic matter in wetland soils and sediments.

Hydrologic regimes that produce seasonal wetting and drying such as rice paddies can provide the driver for iron cycling (Sahrawat, 2004a). During drying, iron species (Fe^{2+}) undergo oxidation and in wetting periods, iron species undergo reduction (Fe^{3+}). Thus, iron cycling has the potential to both limit carbon oxidation and limit methane release (by acting as a subsurface oxidative agent and a competing electron acceptor in microbial respiration).

Net retention of organic matter and plant debris can be observed in most wetlands (Mitsch and Gosselink, 2007). Depending predominantly on the meteorological and hydrological conditions, wetlands can absorb carbon dioxide from the atmosphere and capture it within the sediment and may therefore be greenhouse gas sinks.

Long periods of soil submergence under rice cultivation promoted the formation of passive pools of SOC (Mandal *et al.*, 2008). They reported that 29 per cent of the compost C applied to the soil was stabilized into recalcitrant C pools.

2.2.2.3. Significance of conventional rice cropping system

Rice is primarily cultivated under submerged condition in various parts of the country including irrigation command areas. Since, rice soils are covered with a film of water in major part of the cropping season, it is widely believed that carbon gets locked up in the soil which serves a carbon conservation strategy. The literature pertinent to carbon dynamics in conventional system of rice cultivation is reviewed in this section.

Witt *et al.* (2000) documented the capacity of continuous irrigated rice systems to sequester C and N during relatively short time periods. They reported that 11-12 per cent more C sequestration and 5-12 per cent more N accumulation in soils continuously cropped with rice than in the maize-rice rotation within a period of 2 years.

Sahrawat *et al.* (2005) investigated eight sites in the semi-arid tropical regions of India to evaluate and compare the long-term effects of lowland rice and non-rice (arable systems) on SOC, SIC and total N status. They concluded that continuous wetland rice cropping accumulate organic matter and contain higher soil organic matter compared to the sites under other arable systems.

Long-term rice cropping resulted in substantial increases in SOC contents, even in unfertilized plots compared to the initial level, indicating the potential for SOC sequestration in paddy fields (Yan *et al.*, 2007). They reported that the unfertilized plots contained 27.4 per cent C and 10.5 per cent N more than the original level in a paddy soil.

The impact of long-term intensive rice-rice cropping system with 36 years of application of compost and inorganic fertilizer application resulted in 32 per cent increase in SOC content over the control treatment (Nayak *et al.*, 2009).

Huang *et al.* (2010) recently reported that long-term rice cropping following 27 year differential fertilization regimes increased SOC concentrations by 28.8, 30.1, 30.8, and 61.6 per cent in the non-fertilized, nitrogen, NPK and NPK combined with farmyard manure treatments, respectively.

2.2.2.4. Possibilities of carbon sequestration in system of rice intensification (SRI)

System of Rice Intensification (SRI) was developed during early 1980s in Madagascar by a French priest, Fr. Henri De Laulanie. The SRI includes a set of management practices that are believed to be responsible for higher productivity in comparison to conventional system of rice cultivation. The Tamil Nadu agricultural University is one of the early birds in introducing SRI as a strategy to augment rice productivity in the state of Tamil Nadu. The SRI possesses a set of agronomic practices namely transplanting 14 day old seedlings, square planting (25 x 25 cm), criss-cross conoweeding four times with an interval of 10 days and scheduling irrigation with intermittent wetting and drying conditions upto panicle initiation (Thiyagarajan *et al.*, 2009).

The System of Rice Intensification (SRI) has been promoted for more than a decade as a set of agronomic management practices for rice cultivation. SRI enhances yield (Ceesay *et al.*, 2006; Kabir and Uphoff, 2007; Zhao *et al.*, 2010), reduces water requirements (Satyanarayana *et al.*, 2007), raises input productivity (Sinha and Talati, 2007), accessible to smallholders (Stoop *et al.*, 2002) and is more favorable for the environment than conventional practice with its continuous flooding of paddies (Uphoff, 2003).

a. Factors favouring carbon sequestration processes in SRI

One of the key components of SRI is conoweeding that aerates the rhizosphereic soil which in turn promotes intense microbial activities besides proliferating root growth. Rhizosphere microorganisms are efficient C sequester converting atmospheric CO₂ into biomass carbon. Gyathry (2002) reported that rhizosphere soils of SRI had higher enzyme activities viz., dehydrogenase, urease, acid phosphate, alkaline phosphate and nitrogenase than conventional system. The SRI strategy for weed control is to use a simple mechanical hand weeder in a criss-cross pattern. This implement churns up the top 3–5 cm of soil and buries weeds. When these weeds decompose, this provides additional nutrients to the plants and beneficial aerobic microorganisms (Thiyagarajan *et al.*, 2005). Interestingly, the SRI soils registered higher microbial biomass carbon (MBC) in the range of 2–41 per cent in three of the four seasons (Rupela *et al.*, 2006).

Sudhalakshmi (2002) reported that conoweeding is the key component contributing nearly 40 per cent towards the yield increase in SRI system of cultivation.

Such enhance productivity is closely associated with root proliferation and intense microbial activity.

Roots are the prime location of C accumulation that would significantly contribute for the stable C pool in rice ecosystem. Barison (2003) reported that SRI plants had considerably greater root length density (RLD) in the lower soil horizons compared with conventionally plants. RLD for SRI plants were 2.3 times more at 30–40 cm depth and 3.8 times more at 40–50 cm. They also had greater root-pulling resistance (RPR) as SRI plants at the panicle initiation stage required 7.2 times more force per plant than rice grown with farmer methods. At maturity, this disparity had increased to 14.2 times. Such large differences in root growth are the most evident phenotypical differences induced by SRI practices. Rupela *et al.* (2006) observed that SRI plants had about 10 times more root mass, about five times more root length density and about seven times more root volume in the top 30 cm of soil profile compared with roots of conventional rice plants. They also reported that the root length in the top 15 cm of the soil were 19.8 km m³ and 2.4 km m³ for SRI and conventional system, respectively. SRI practices in particular produce much larger and longer-lived root systems and these are associated with increased and modified populations of soil biota (Uphoff *et al.*, 2009).

In SRI, irrigation is scheduled to maintain the moisture content near field capacity. This helps to reduce the CH₄ emission from the rice field's vis-à-vis global warming impacts. It has been estimated that CH₄ emission is 40 per cent lower in SRI compared to submerged rice cultivation (Jayadeva *et al.*, 2009).

One of the remarkable reports of SRI is the production of highest number of tillers ranging from 60 to 80 and even more per hill (de Laulanie, 1993). As SRI plants produce enormous quantities of biomass, more C gets accumulated in the tillers through efficient photosynthesis. Uprety (2004) reported that the average rice yield with SRI as 8 t ha⁻¹, whereas the yield is 3 t ha⁻¹ under conventional system. Uphoff (2002, 2004) reported the average rice yield with SRI to be double the current average yield and at times, the SRI yields upto 21 t ha⁻¹. Sinha and Talati (2007) evaluated 167 on-farm trials in Andhra Pradesh and reported average yield obtained using SRI practices to be 8.1 t ha⁻¹, compared with 5.67 t ha⁻¹ using conventional practices.

Vijayakumar (2003) evaluated the productivity of SRI in comparison with conventional practices. The SRI components such as planting 14 days old younger seedling at a spacing of 25 x 25 cm adopting water saving irrigation schedule (intermittent wetting and drying) with rotary weeding contributed for highest production. It was concluded that set of these SRI practices demonstrated highest grain yield, number of tillers, total dry matter production, root length, root volume and root dry weight.

Senthilkumar *et al.* (2008) reported that SRI contributed for 22 per cent higher grain yield over normal transplanting. They also indicated that SRI had highest number of tillers in unit area, total biomass and harvest index.

Sathiya and Sathyamoorthi (2009) reviewed the effectiveness of individual SRI components in comparison to conventional practices. They concluded that SRI components have outperformed the conventional recommendations with respect to growth and yield attributes.

Dumas-Johansen (2009) evaluated the effect of SRI on the farmers' livelihood situation and reported through theoretically calculations that SRI inherits significant potentials for carbon storage and mitigation of greenhouse gases.

Thakur *et al.* (2010) assessed the physiological effects of SRI practices in comparison with Recommended Management Practices (RMP). SRI spacing used was 20 per cent less than usually recommended and accordingly, they reported the results as a modified SRI (SRI_m). They observed that SRI_m out-yielded RMP by 42 per cent. The number of tillers per hill in SRI_m varied from 13 to 36 (average: 17.9 tillers hill⁻¹) whereas in RMP the number ranged from 6 to 16 (average: 9.7 tillers hill⁻¹). On the other hand, tiller number per unit area was significantly lower in SRI_m than in the RMP plots. The dry weight of aboveground parts was greater in SRI_m than RMP when compared per hill, but no significant difference was found in dry matter production when the two methods of cultivation were compared on a unit area basis. The straw weight per unit area was significantly higher in RMP than SRI_m plots. Further, harvest index was significantly higher for SRI_m than RMP. RMP produced 1.6 μ mol CO₂ fixed per m mol water transpired, compared to 3.6 μ mol CO₂ in SRI_m plants. They concluded that higher photosynthesis rate coupled with lower transpiration in SRI_m plants indicated that they were using water more efficiently than RMP plants.

Despite the fact that the SRI is known to promote rice productivity irrespective of agro-climatic zones across the globe, there is a bundle of conflicting reported literatures suggesting extensive research efforts required to explore the enigmatic advantages of SRI cultivation. Over the recent years an intense debate has been going on among some scientists about the possible merits of the SRI system as compared with the conventional rice production system (Stoop *et al.*, 2009). SRI reports of yield benefits and phenotypical changes with SRI management have been challenged on various grounds (Dobermann, 2004; Sheehy *et al.*, 2004; Sinclair and Cassman, 2004; Latif *et al.*, 2005; McDonald *et al.*, 2006).

For instance, Sheehy *et al.* (2004) based on a theoretical model for predicting maximum yields attainable and on the results from a few field trials, concluded that the reported high yields under SRI must be due to measurement errors and that „SRI has no major role in improving rice production generally’. SRI results have been characterized by Sinclair (2004) and Sinclair and Cassman (2004) as „unconfirmed field observations’, with the admonition that SRI offers no shortcuts to achieving large yield increases. Commenting on the effects of SRI practices, Sinclair (2004) contended that SRI’s very low plant densities would lead to poor light interception, whereas high plant density is a prerequisite for enhanced light interception, growth and yield.

Sheehy *et al.* (2004) conducted experiments in three locations in China comparing yields in conventional and SRI management systems. In addition, they used a theoretical model to predict the maximum yields and compared those with reported yields for various locations, including China and Madagascar. They remarked that the SRI has no inherent advantage over the conventional system and that the original reports of extraordinary high yields are likely to be the consequence of error.

Dobermann (2004) accepted SRI as possibly a „niche innovation’ for improving rice production on poor, acidic soils with potential for Fe toxicity, but asserted it had little potential for improving yield in intensively irrigated systems with more favourable soils.

Latif *et al.* (2005) compared the performance of SRI, existing best management recommendations and farmer’s practices on rice yield. SRI practice yielded an average of 5.95 t ha⁻¹ while, best management recommendation and farmers’ practices resulted in

6.88 and 5.05 t ha⁻¹, respectively. They suggested that several of the key management principles stated in SRI had in fact little effect on rice yields. Moreover, SRI did not increase rice yields, when it was compared to existing best management practices.

McDonald *et al.* (2006) evaluated the productivity of SRI with respect to conventional best management practices (BMP) using 40 site-years database. In addition to data from Madagascar where SRI was first conceived, findings from a broad geographic region were compiled including studies from Nepal, China, Thailand, Laos, India, Sri Lanka, Indonesia, Bangladesh and Philippines. Aside from one set of experiments in Madagascar where SRI more than doubled rice productivity with respect to BMP, they found no evidence of a systematic or even occasional yield advantage of this magnitude elsewhere. Indeed, none of the 35 other experimental records demonstrated yield increases that exceeded BMP by more than 22 per cent. Excluding the Madagascar examples, the typical SRI outcome was negative, with 24 of 35 site-years demonstrating inferior yields BMP. They concluded that there is no evidence in the empirical record that SRI fundamentally changes the physiological yield potential of rice.

Sheehy *et al.* (2005) argued that the energy requirements for achieving such high yield in SRI are beyond the thermodynamic capabilities of rice plants' photosynthesis and the crop's use of solar energy.

The debate has been focused on comparison of SRI performance with available „best management practices' (BMPs) (McDonald *et al.*, 2008 and Uphoff *et al.*, 2008). McDonald *et al.* (2008) challenged the scientific basis of supporting reports of SRI's remarkable yield performance furnished by Uphoff and firmly concluded that there is still no evidence that the SRI out-yields best BMPs beyond Madagascar.

Overall, the data suggest that SRI has the great potential to improve the grain and straw yields of rice compared to the conventional systems with distinct advantages in carbon stock as a result of management practices that favours both biological activities and resilient carbon pools. This makes the thesis very unique and innovative to look at carbon sequestration in two distinct systems of rice cultivation with impetus to environmental benefits associated with SRI.

2.3. Methane emissions from Paddy fields

Rice fields are an important atmospheric methane (CH_4) source, contributing about 5–19 per cent of total global CH_4 emissions to the atmosphere (IPCC, 2007). Methane is the important greenhouse gas next to carbon dioxide, which is 25 times greater in global warming potential than CO_2 on a 100-year horizon (IPCC, 2006). When the 18th century Italian physicist Alessandro Volta first identified methane from a waterlogged marsh, he could not have guessed how important this gas would prove to human society in the centuries that followed (Reay *et al.*, 2010). Recently, Uprety *et al.* (2011) reviewed the methane emissions in rice fields and reported the future emission scenarios. The methane emissions in 2030 in India are projected to reach 24.4 Tg (reference scenario), 21.3 Tg (medium mitigation scenario) and 17.6 Tg (strong mitigation scenario).

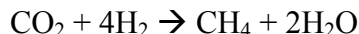
The first field measurements of CH_4 emissions from rice paddy fields were conducted in California by Cicerone and Shetter (1981), followed by extensive studies in India (Adhya *et al.*, 1994; Ramakrishnan *et al.*, 1995; Banik *et al.*, 1995; Debnath *et al.*, 1996; Satpathy *et al.*, 1997). In spite of these intensive efforts to obtain reliable field data, the available database still lacks direct measurements in several agro-climatic regions. Furthermore, a region-specific characterization of agronomic (fertilizer application, water management, etc.) and natural factors (soil properties, weather patterns, etc.) are indispensable to cope with the extreme temporal and spatial variability in CH_4 emission from the same rice cultivation systems (Minami, 1995) or different rice ecosystems (Wassmann *et al.*, 1997). Hence, the diversified conditions in crop management and environments for growing rice are not sufficiently characterized for accurate estimates (Byrnes *et al.*, 1995; Wassmann *et al.*, 1998).

2.3.1. Mechanism of Methane production in rice soils

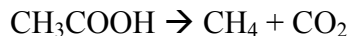
Harrison and Aiyer (1913) did pioneering work on the gases liberated in rice fields. Acharya (1935a and 1935b) observed that in wetland rice fields, organic matter is degraded to the gaseous end-products such as CO_2 and CH_4 . Methane is produced as the terminal step of the anaerobic decomposition of organic matter in wetland soils (Sahrawat, 2004a).

The two major pathways that produce CH₄ in submerged soils (Takai, 1970; Neue and Scharpenseel, 1984) include:

- i. Reduction of CO₂ with H₂ (deriving from an organic compound)



- ii. Decarboxylation (transmethylation) of acetic acid



Various factors such as temperature, soil pH, and addition of organic materials influence the ratio between CH₄ and CO₂ produced (Aulakh *et al.*, 2001b). Methane production requires flow of carbon and electrons to microbial population of methanogens under reduced conditions in the strict absence of free oxygen (Sahrawat, 2004a). It is produced in rice fields after the sequential reduction of molecular oxygen, nitrate, iron (III), manganese (IV) and sulfate, which serves as electron acceptors for oxidation of organic matter to CO₂ (Ponnamperuma, 1972). The final products of reduction in submerged soils are Fe (II) from Fe (III), H₂S from SO₄⁻² and CH₄ from CO₂. In rice soils, root exudates provide important C sources for CH₄ production. On an average, 30–60 per cent of photosynthesized C by plants is allocated to the roots and a substantial portion of this C is released or secreted by roots in the form of organic compounds in the rhizosphere (Marschner, 1995). The organic acids in root exudates supply energy to soil microbial communities including methanogens (Aulakh *et al.*, 2001b).

(i) Influence of soil physical and chemical properties

The CH₄ production potential of soils is an important component for understanding site-to-site variations in CH₄ budgets (Aulakh *et al.*, 2001b). Methane production in reduced soils is very sensitive to pH with an optimum pH range from 6.7–7.1 (Jakobsen *et al.*, 1981; Bouwman, 1990) that varies with soil type. For example, methanogenesis was found optimum in a pH range of 7.5–8.5 in four different Indian soils (Parashar *et al.*, 1991). In general, flooding of soil causes the soil pH to stabilize between 6.5 and 7.2 (Ponnamperuma, 1972), which is the optimum range for CH₄ production. High pH in the water-soil interface may also inhibit CH₄ oxidation (King *et al.*, 1990).

Soil anaerobiosis is measured in terms of redox potential (Eh), ranging from about -100 to -200 mV has been reported to be needed for the initiation of CH₄ production in paddy soils (Takai, 1961; Cicerone *et al.*, 1983; Lindau *et al.*, 1991).

As texture determines several physical and chemical properties of the soil, it could influence CH₄ emission indirectly. For instance, percolation rate, directly related to the soil texture, affects CH₄ production in paddy soils (Yagi *et al.*, 1990). The temperature dependency of CH₄ production rates has been shown for several soils (Seiler *et al.*, 1984; Parashar *et al.*, 1991). Methane flux rates were dependent on soil temperature at 5 cm depth and emissions doubled as soil temperature increased from 20–25°C (Holzapfel-Pschorn *et al.*, 1986). Studies in India showed that CH₄ fluxes increased as soil temperature increased to 35°C but emissions decreased above this temperature (Parashar *et al.*, 1993).

(ii) Influence of soil biology and biochemistry

Methane is produced in the anaerobic layers of paddy soil by bacterial decomposition of organic matter (Dubey, 2001). The anaerobic degradation of organic matter involves four main steps: a) hydrolysis of polymers by hydrolytic organisms, b) acid formation from simple organic compound by fermentative bacteria, c) acetate formation from metabolites of fermentations by homoacetogenic or syntrophic bacteria, and d) CH₄ formation by methanogens from H₂/CO₂, acetate, simple methylated compounds or alcohols and CO₂ (Conrad, 1996; Yao and Conrad, 2001). Hence, the methanogenic degradation of organic matter in flooded soils is achieved by a complex microbial community consisting of hydrolytic, fermenting and methanogenic microorganisms (Conrad, 2007).

a. Methanogens

Methanogens exclusively produce methane in the strict absence of free oxygen and at redox potentials of less than -150 mV (Wang *et al.*, 1993). They are strictly anaerobic unicellular organisms originally thought to be bacteria but now recognized as belonging to a separate phylogenetic domain, the *archae* (Garcia, 1990). Methanogens mainly use acetate (contributes about 80 per cent to CH₄ production) as a carbon substrate but other substrate like H₂/CO₂ and formats also contribute 10-30 per cent to CH₄ production (Chen *et al.*, 1993). All methanogens use NH₄⁺ as a nitrogen source,

although the ability to fix molecular nitrogen and the *nif* gene is present in all the three orders (*Methanobacteriales*, *Methanococcales* and *Methanomicrobiales*) of methanogens (Palmer and Reeve, 1993).

The methanogens are the best at work at a pH of 6–8 and a temperature of 30–40°C (Vogels *et al.*, 1988). Methane is usually produced by acetoclastic and hydrogenotrophic methanogenic archaea. In general, CH₄ production was at first dominated by hydrogenotrophic methanogenesis which subsequently decreased as acetoclastic methanogenesis became progressively more important and finally increased again (Conrad, 2007). Rice field soils frequently exhibit a rather large diversity of such methanogenic archaea, including acetoclastic *Methanosarcinaceae* and *Methanosaetaceae* as well as hydrogenotrophic *Methanocellales*, *Methanomicrobiales* and *Methanobacteriales* (Conrad *et al.*, 2012).

The mechanism of methanogenesis in paddy fields worldwide has been investigated in detail. However, information regarding methanogenic population size in rice fields is limited (Dubey, 2005). Rajagopal *et al.* (1988) were the first to carry out isolation and characterization of methanogens from Louisiana paddy fields and reported the presence of two *Methanobacterium*-like strains and two *Methanosarcina*-like strains. Kudo *et al.* (1997) reported the presence of *Methanosarcina*, *Methanogenium*, *Methanosaeta* and *Methanoculleus*-like organisms in rice paddy fields. Recently Ma *et al.* (2011) reported that the abundance of methanogens in rice field soils is usually rather high ($>10^6$ cells g⁻¹ dry soil) throughout the season, even when the soil is drained.

b. Methanotrophs

The amount of CH₄ emitted from rice fields to the atmosphere is the balance of two opposite processes, i.e., CH₄ production and oxidation in the soil. In the global CH₄ cycle, substantial amount of CH₄ is consumed by biological processes. The only known biological sink for atmospheric CH₄ is its oxidation in aerobic soils by methanotrophs or methane-oxidizing bacteria (MOB), which can contribute up to 15 per cent to the total global CH₄ destruction (Singh, 2011).

Methanotrophic bacteria are strictly obligate aerobes and use monocarbon organic compounds such as CH₄ and methanol as a substrate (Papen and Rennenberg, 1990). As they require molecular O₂ (Bedard and Knowles, 1989), methanotrophs are active in

oxic - anoxic interfaces where concentration gradients of CH_4 and O_2 overlap. Therefore, a sizable fraction of CH_4 produced in the reduced soil matrix or in micro-sites is oxidized when it diffuses upward. Methanotrophs oxidize CH_4 with the help of methane monooxygenase (MMO) enzyme. MMO catalyze the reaction to convert CH_4 , O_2 and reducing equivalents to methanol and H_2O . The MMO occurs in two forms: as a membrane bound particulate form (sMMO) in all types of methanotrophs and as a soluble form in Type-II and Type X methanotrophs (Mancinelli, 1995).

Methanotrophs are very sensitive to environmental perturbations and therefore, the factors that limit or even inhibit the activities of MOB may have major effects on the global CH_4 budget. Besides major limiting factors, i.e. CH_4 and oxygen, nitrogen is one of the limiting factors for CH_4 oxidation and may become an inhibiting or stimulating factor for growth of methanotrophs (Singh, 2011).

Inorganic N influences CH_4 oxidation due to shifts in the population structure and the kinetics of methanotrophs (Dubey *et al.*, 2002). This may affect the threshold value for CH_4 oxidation (King, 1992). NO_3^- -N fertilization did not affect the CH_4 consumption but NH_4^+ -N fertilization completely ceased CH_4 oxidation (Hutsch *et al.*, 1994). However, nitrite was found to inhibit CH_4 oxidation in the cultures of *Methylobacterium albus* BG8 and *M. trichosporium* OB3b (King and Schnell, 1994). Currently, there are many contradictory results reporting inhibition effects, stimulation effects or no effects of NH_4^+ -N based nitrogen fertilizers on MOB. So far, many mechanisms have been proposed for stimulation and inhibition effects on methane oxidation and methanotrophs, but none of them has yet been experimentally verified (Singh, 2011).

2.3.2. Pathways of Methane transport

Methane escapes from the paddy soils to the atmosphere by three pathways (Fig. 2.2) viz. ebullition, diffusion and transport through the rice plant (Dubey, 2005). These pathways are briefly discussed below.

(i) Ebullition

Ebullition (gas transport *via* gas bubbles) is a physical process through which methane escapes from the soil in the form of bubbles. Ebullition of CH_4 from sediments is a common and significant mechanism accounting for 49–70 per cent of the total flux in

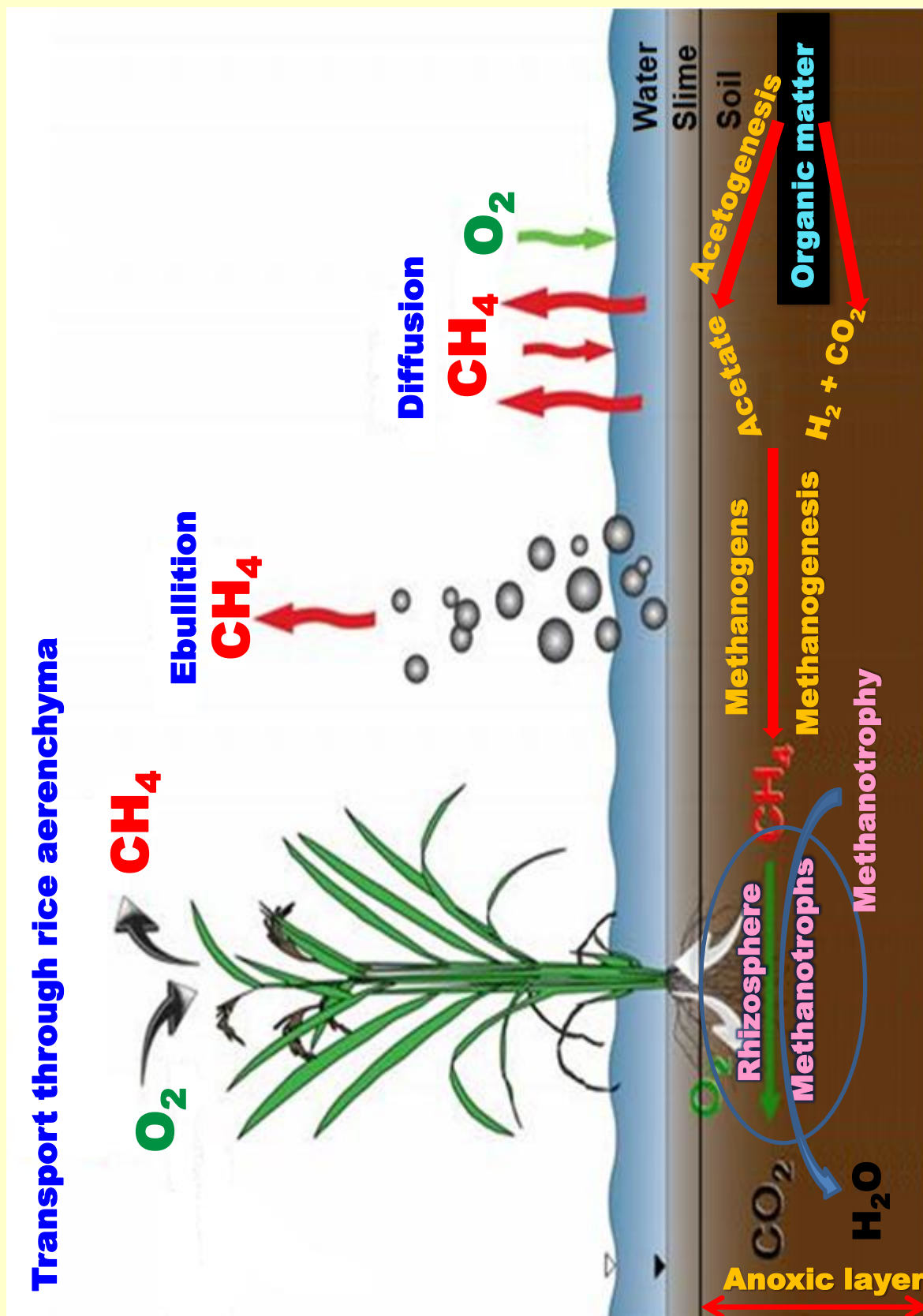


Fig.2.2. Schematic diagram of methane production, oxidation and emission

natural wetlands (Wassmann and Martius, 1997). However, in rice fields CH_4 ebullition is a predominant mechanism only during the initial plant growth stage and during cultural operations (Wassmann *et al.*, 1996). Disturbance of soil due to intercultural operation in the rice field could release soil entrapped CH_4 and enhance its emission through ebullition (Denier van der Gon *et al.*, 1992). If soil is unvegetated or plant aerenchyma is not yet well-developed, ebullition plays a major role in CH_4 emission (Byrenes *et al.*, 1995) but it occurs only at surface layer and its rate is regulated by CH_4 concentration, temperature, soil porosity and plant aerenchyma (Li, 2000).

(ii) Diffusion

Diffusion of CH_4 across the flooded soil and overlying water of the rice field to the atmosphere is a function of surface-water concentration of CH_4 , wind speed and CH_4 supply to the surface-water (Sebacher *et al.*, 1983). The transport of CH_4 via diffusion through soil and water is very small. Its contribution to total CH_4 emission during the rice growing season is often 1–2 per cent (Butterbach-Bahl *et al.*, 1997). Produced CH_4 is oxidized, especially in the soil-water interface and some in the rhizosphere of the rice plant. Therefore, CH_4 diffusion contributes only a small amount to the total CH_4 flux from rice fields. Moreover, CH_4 diffusion through the soil is a very slow process because the „diffusion rate’ of gaseous CH_4 is very low in liquid phase (about 10^4 times slower than diffusion through the gas phase) and hence, it hardly contributes to the total CH_4 flux (Aulakh *et al.*, 2001b).

(iii) Transport through rice plants

Plant mediated transport is the primary mechanism (Wassmann *et al.*, 2000) for the CH_4 emission from paddy fields and contributes 90 per cent to the total CH_4 flux (Khosa *et al.*, 2010 and Akinbile *et al.*, 2012). As a morphological adaptation to flooding, hydrophilic and wetland plants including rice possess aerenchyma. This air-filled tissue is required to aerate the roots of hydrophilic and wetland plants and constitutes the major pathway for the flux of gases through plants into the roots (Aulakh *et al.*, 2001b).

Methane dissolves in the soil-water surrounding the roots and diffuses into the cell-wall water of the root epidermis cells and then diffuses through the cell-wall water of the root-cortex, depending upon the concentration gradient between the soil-water

surrounding the roots and the lysigenous inter-cellular spaces in the roots. Methane is then gasified in the root cortex and transported to the shoots *via* lysigenous intercellular spaces and aerenchyma. Eventually, CH₄ is released primarily through the micropores in the leaf sheath of the lower leaf position and also through the stomata in the leaf blade (Nouchi *et al.*, 1990). In the overall methane dynamics, rice plants act as: a) source of methanogenic substrate, b) conduit for CH₄ through well developed system of inter cellular air space (aerenchyma) and c) potential methane oxidizing micro-habitat in the rhizosphere by diffusing oxygen which favour the growth and multiplication of methanotrophs (Dubey, 2005).

2.3.3. Strategies for methane mitigation

Irrigated rice represents the most promising target for methane mitigation strategies (Aulakh *et al.*, 2001b). The most reliable mitigation options without affecting yield are discussed below.

(a) Water management

Water management of rice fields is crucial for CH₄ emission because the development of an anaerobic environment in soils results from limitation of O₂ supply from the atmosphere by flooding the soils. Mid-season drainage, intermittent irrigation or pre-harvest field drying could be effective in reducing CH₄ fluxes (Buendia *et al.*, 1998). These management practices appear to be useful no-regret options for reducing CH₄ emissions without reducing rice yields. During the dry spells, an increase in soil Eh and a decrease in CH₄ flux are often observed resulting in markedly reduced total seasonal CH₄ emissions (22–88 per cent) compared to the continuously flooded treatment without reducing rice yields (Mishra *et al.*, 1997; Jain *et al.*, 2000).

Compared with continuous flooding, intermittent irrigation and constant moisture (field with no standing water, but remained saturated) reduced methane emission rate by 25.4 and 58.4 per cent, respectively (Wang *et al.*, 1999). Methane emission was $15.27 \pm 1.46 \text{ g m}^{-2}$ with intermittent irrigation and it was $28.85 \pm 3.25 \text{ g m}^{-2}$ with continuous flooding. The average methane emission rate was 5.3 ± 0.56 and $9.54 \pm 1.07 \text{ mg m}^{-2} \text{ h}^{-1}$ for intermittent irrigation and continuous flooding, respectively.

Wassmann *et al.* (2000) reported that the distinct drainage periods within the season can drastically reduce CH₄ emissions to less than 30 kg CH₄ ha⁻¹ season⁻¹ and such water management practices could reduce CH₄ emission without affecting yields. Mean CH₄ emission was lowest (13.80 mg m⁻² d⁻¹) in field plots that were alternately flooded as compared to continuously flooded (16.32 mg m⁻² d⁻¹) plots leading to a 15 per cent reduction in seasonal CH₄ flux (Adhya *et al.*, 2000).

Intermittent irrigation system for paddy fields reduces the water consumption in rice cultivation and mitigates methane emission in agriculture production (Yang and Chang, 2001). Aerating the soil during the flooding periods with altered water management leads to decrease in the methane production rate from the soils. Since methanogens require highly reducing conditions, aerating the soils arrests the development of reducing conditions in the soils and enhances the methane oxidation, which leads to a decrease in methane production level and ultimately to a total decrease in the methane emissions. Thus, proper drainage during the growing season could be a promising mitigation strategy that should not affect yields substantially (Anand *et al.*, 2005).

Setyanto and Bakar (2005) investigated the effect of four water regimes on methane emissions. The treatment comprised of 5 cm continuous flooding (CF), 0-1 cm continuous flooding (ST), intermittent irrigation (IR) where plots received continuously 5 cm of flooding with two times of draining at 15-20 and 25-30 days after transplanting (DAT), and pulse irrigation (PI) where plots were watered until 5 cm level and left to dry by itself until the water table reached 30 cm beneath soil surface then watered again. The total CH₄ emissions of the four water treatments were 254, 185, 136 and 96 kg CH₄ ha⁻¹ for CF, ST, IR and PI, respectively. This study suggested that intermittent and pulse irrigation practices will be important not only for water use efficiency, but also for CH₄ emission reduction.

During the Alternate Wetting and Drying (AWD) cycle, most of the methane gas oxidizes before reaching the soil – air interface resulting in lower methane flux (Purkait *et al.*, 2007). The soil Eh during intermittent drainage was higher than that of continuously flooded period particularly at the mid-season aeration periods. High Eh values prevented CH₄ formation by methanogenic bacteria and hence reduced the CH₄ emissions during intermittent drainage (Hadi *et al.*, 2010).

Li *et al.* (2011) conducted experiments with four different moisture regimes namely early aeration, normal aeration (the same as the local practice in timing and duration of aeration), delayed aeration and prolonged aeration to evaluate the methane emission potentials. Compared with the local practice, early aeration reduced CH₄ emission by 13.3–16.2 per cent, while delayed aeration increased CH₄ emission by 22.1– 47.3 per cent. The lowest CH₄ emissions occurred in prolonged aeration treatment. However, rice grain yield was reduced by 15.3 per cent when compared with normal practice. They observed that midseason aeration starting around 30 days after rice transplanting would optimize rice yields while simultaneously limiting CH₄ emissions. They concluded that early aeration strongly reduces CH₄ emission from rice paddy fields.

Recently, Khosa *et al.* (2011) investigated the effects of six different water regimes on methane emissions. The plots were continuously flooded for first two weeks and then irrigated under six different water regimes viz. continuous flooding, alternate flooding and draining and the remaining treatments were irrigated when matric potential reached the value of 0.10, 0.15, 0.20 and 0.30 bar. The matric potential was measured by ceramic porous cup tensiometer fitted at 15 cm soil depth. Results showed that compared to continuous flooded conditions, the seasonal methane flux could be reduced by 38 and 61 per cent by applying irrigation at 0.10 and 0.15 bar matric potential without affecting the grain yield. They reported that by irrigating the field at 0.15 bar matric potential reduced seasonal methane flux by 60 per cent as compared to completely flooded conditions.

(b) Inorganic fertilization

Nutrients essential for plant growth, either naturally occurring or added through fertilizers to paddy soil may affect the production and emission of CH₄ by influencing (a) the growth of the rice plant, (b) the activity of methanogenic microbial communities and (c) the amount and composition of root exudates of growing rice plants (Aulakh *et al.*, 2001a). Numerous studies have revealed the impact of chemical fertilizers on CH₄ emissions (Schutz *et al.*, 1989., Adhya *et al.*, 2000; Sethunathan *et al.*, 2000) and among the major nutrients, the effects of nitrogen fertilizers on methane emissions are still inconclusive (Aulakh *et al.*, 2001b).

(i) Nitrogen

Although the impact of rate, type and method of N application has been investigated in several field studies, the available data are mostly unclear (Cheng-Fang *et al.*, 2012). Reviewing wide range of literatures on this topic suggests that the published results can be classified into the ones that reported positive effects and other set of research data with negative effects of nitrogen on methane emissions.

Several researchers have reported that nitrogen fertilizers inhibited the methane emissions. Schutz *et al.* (1989) reported that the application of 100 and 200 kg urea - N ha⁻¹ to rice fields over a 3-year period reduced CH₄ emissions by 18 per cent as compared to control plots. They reported that the application modes differed in their impact with highest CH₄ flux from “surface applied urea” followed by “raked into soil” (5 cm depth) and “incorporated” (20 cm depth). Lindau (1994) reported the decrease in CH₄ emission rate with ammonium nitrate application due to competitive inhibition of nitrate reduction in favour of methane production.

Udayasoorian (1995) suggested that the ammonium sulphate is an eco-friendly nitrogen fertilizer and reduces methane flux. The data showed that methane emissions were lower in plots that were fertilized with urea, phosphorus and potassium when compared to the plot without nitrogen fertilizers (P and K fertilizer alone) indicating the potential of urea to inhibit methane emission. However, this study also reported that methane emissions were higher in plots that were fertilized with urea, phosphorus and potassium when compared to control plots. This investigation also brought out the complexity of urea fertilizers on methane flux. Cai *et al.* (1997) investigated the effect of ammonium sulphate and urea on methane emissions. Results showed that CH₄ emissions decreased by 42 and 60 per cent in the ammonium sulphate treatments and 7 and 14 per cent in the urea treatments at rates of 100 and 300 kg N ha⁻¹, respectively, compared to the control. They concluded that CH₄ emission would not be due to the effect of sulphate reduction on soil Eh. In addition, they noticed that both depressive and stimulative effects of urea application were observed depending on the changes in NH₄⁺ concentration. The negative effect of ammonium-based fertilizer is mainly attributed to the stimulation on CH₄ oxidation *via* enhancing the growth/activity of methanotrophs (Bodelier and Laanbroek, 2004).

In contrast, Lindau *et al.* (1991) observed that the surface-applied urea at the rates of 0, 100, 200 and 300 kg ha⁻¹ evolved total CH₄ emissions of 210, 300, 310 and 370 kg ha⁻¹, respectively and these results revealed that the urea had a stimulatory effect on methane emission. Lindau and Bollich (1993) also showed enhanced CH₄ emission with surface-applied urea as a result of hydrolysis to ammonium.

Urea application enhances CH₄ fluxes possibly by increasing soil pH following urea hydrolysis and subsequent drop in redox potential, stimulates methanogenic activities (Wang *et al.*, 1993). Addition of ammonium to flooded rice soils has been reported to inhibit CH₄ oxidation (Conrad and Rothfuss, 1991; Wassmann *et al.*, 1993) and hence, may increase the percentage of the CH₄ produced that is emitted into the atmosphere.

Lu (1998) demonstrated that NH₄-N application promoted higher and faster development of dissolved CH₄ concentration and CH₄ emission in flooded rice. The positive effect of nitrogen mainly results from the stimulation of CH₄ production and vascular transport capacity *via* enhancing methanogenic growth/ activity and rice growth (Xu *et al.*, 2004).

Numerous previous studies have demonstrated that the application of nitrogen may directly or indirectly influence all of the processes in CH₄ production, oxidation and transport from the soil to the atmosphere and therefore, the effects of N application on CH₄ emission at the ecosystem level is the net consequence of these processes (Cai *et al.*, 2007).

Ma *et al.* (2007) reported that urea decreased CH₄ emission relative to the control when applied at a rate of 200 kg N ha⁻¹ but the effect lessened if the application rate was further increased to a rate of 270 kg ha⁻¹. They showed that effects of urea application on CH₄ emissions depend on its integrated effects on CH₄ production and oxidation. Since effects of urea application on CH₄ production, oxidation and transport can be negative and positive and take place simultaneously, it is not surprising that the reported effects of urea application on CH₄ emissions from rice fields are contradictory.

Xie *et al.* (2010) investigated across various climatic zones and reported that the application of ammonium-based fertilizers at widely applied rates usually inhibits CH₄ emission as compared to no N addition. Ammonium-based fertilizer at the rate of 150 kg N ha⁻¹ greatly inhibited as compared to fields without N application. However,

the inhibitory effect was not observed by further increasing N application to 250 kg N ha⁻¹. Out of the ten cases, seven cases recorded the methane reduction by 14 per cent whereas remaining three cases registered an enhancement of methane emission by 47 per cent. However, they reported that all of these inhibitory or stimulatory effects were not statistically significant. Hence, they concluded that further studies are very much necessary (Xie *et al.*, 2010).

Recently, Cheng-Fang *et al.* (2012) reported that the application of nitrogen fertilizer significantly increased CH₄ emissions by 13 – 66 per cent and attributed that nitrogen promoted the rice growth and in turn, provided additional C sources to methanogenic communities.

Further experiments with more nitrogen application rates and simultaneous measurements of CH₄ production, oxidation and emission are necessary to help elucidate the underlying mechanisms (Ma *et al.*, 2007) and to bring out a clear relationship between them.

(c) System of cultivation on methane emission

Combining individual mitigation options to packages of technologies can effectively reduce emissions (Aulakh *et al.*, 2001b). Jayadeva *et al.* (2009) investigated the effect of three systems of rice cultivation viz., Transplanting, System of rice intensification (SRI) and Aerobic on methane emissions. Results indicated that the SRI significantly recorded higher methane emission during early stages of crop growth than normal transplanting while aerobic system registered lowest methane flux. However, at later stages the normal transplanting recorded higher methane emission compared to SRI. The increased methane emission in SRI at initial stages (30 DAS) was attributed to planting of young seedlings at wider spacing (25 cm x 25 cm) under puddled condition, which lead to large root volume besides profuse and strong tillers resulted in more biomass and increased release of methane. The methane emission at 40 and 50 DAS was on par between SRI and normal transplanting. At later stages (60 and 70 DAS) of crop growth the methane emission was significantly higher in normal transplanting. Over all, the total methane emissions were found to be higher in normal transplanting system of rice cultivation. They concluded that increase in methane emission under normal transplanting might be due to methanogenesis as a result of flooding.

The literature review suggests that System of rice cultivation has an impact on methane emission and associated global warming effects. Since, SRI cultivation carries a series of conoweeding practices during the early stages of crop growth that aerates the soil, the methane emission is circumvented. Further, the maintenance of wetting and drying during the initial stages of crop cultivation inhibits methane emission from rice fields. Though these two factors are known to minimize methane emission, there is no systematic study undertaken to precisely relate intercultural operations and methane emission. This thesis has taken assiduous efforts to address the methane emission in relation to conoweeding so as to harness the benefit of SRI cultivation as a strategy to reduce the methane emission and ensure environmental safety.

2.4. Response of rice to elevated carbon dioxide concentrations [CO₂]

Carbon emissions related to human activities have been significantly contributing to the elevation of atmospheric [CO₂]. More recently, atmospheric carbon dioxide concentrations have greatly accelerated, thus much stronger effects on crops are expected. Projections to the end of this century suggest that atmospheric [CO₂] will top 700 ppm or more (IPCC, 2007). Photosynthesis is a major process of sequestration and turnover of the total carbon on the planet. Crops sense and respond directly to rising [CO₂] through photosynthesis and stomatal conductance and this is the basis for the CO₂ fertilization effect on crop yield (Long *et al.*, 2006). There is growing evidence suggesting that C₃ crops are likely to produce more harvestable products and that both C₃ and C₄ crops are likely to use less water with rising atmospheric [CO₂] in the absence of stressful conditions (DaMatta *et al.*, 2010).

The direct effects of rising CO₂ on plant growth and metabolism are a modulation of stomatal conductance, changes in carboxylation capacity and accumulation of photoassimilates. These three regulatory mechanisms will have a wide range of indirect effects on growth and development of plants (Fig.2.3). Other factors which can also influence plant responses to elevated CO₂ are the growth environment, soil nutrition and the genetic organization of the plant species. The up and down-regulation of photosynthates under elevated CO₂ is a complex process regulated by morpho-physiological changes associated, during plant growth and development, with particular reference to carbon allocation between source and sink tissues.

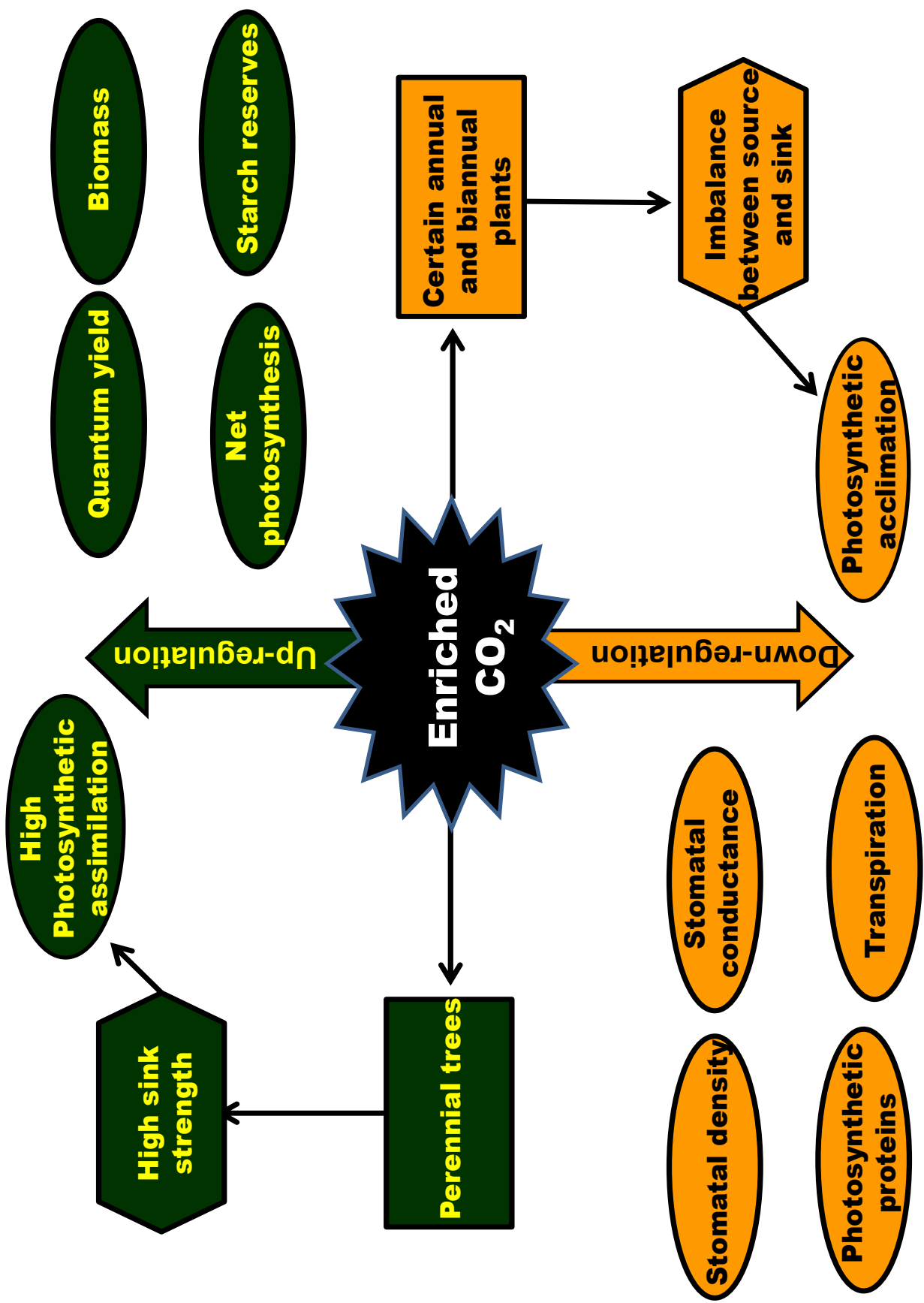


Fig. 2.3. Effects of elevated $[\text{CO}_2]$ on the regulation of plant growth and metabolism
(Modified from Reddy *et al.*, 2010)

2.4.1. Physiological responses to elevated [CO₂]

The bulk of vegetation belongs to the C₃ photosynthesis group. This group is called C₃ because the „first’ product of carboxylation is a 3-carbon acid, phosphoglyceric acid (PGA) (Benson, 2002 and Bassham, 2003). Carbon is assimilated through the reductive pentose phosphate cycle, often referred to as the Calvin cycle. In the first step of this cycle, one molecule of CO₂ reacts with a molecule of ribulose-1,5-bisphosphate (RuBP) to yield two molecules of 3-phosphoglycerate, a three-carbon compound. This reaction is catalyzed by the RuBP carboxylase/ oxygenase, referred to as Rubisco. In addition to its function as a carboxylase, Rubisco also act as an oxygenase. The latter function initiates a series of physiological events in which light-dependent O₂ uptake is associated with CO₂ evolution, a process known as photorespiration. Such a process works in opposite direction to photosynthesis, causing a loss of the CO₂ fixed by the Calvin cycle.

Out of 15 crops which supply 90 per cent of the world’s calories, 12 have the C₃ photosynthetic pathway. Reddy *et al.* (2010) reviewed the literature (1994–2009) on the influence of elevated CO₂ among certain C₃, C₄ and crassulacean acid metabolism (CAM) species and suggested that most of the C₃ plants showed a significant positive response. The direct increase in photosynthesis due to elevation of [CO₂] results from two properties of Rubisco of terrestrial C₃ plants (a) The K_m of the enzyme for CO₂ is close to the current atmospheric concentration, so elevated [CO₂] increases the velocity of carboxylation (b) CO₂ competitively inhibits the oxygenation reaction, which produces glycolate leading to photorespiration. This latter effect is particularly important because it increases the efficiency of net carbon CO₂ uptake by decreasing photorespiratory CO₂ loss and diverting ATP and NADPH (generated by the light reactions) away from photorespiratory metabolism to photosynthetic assimilation. Thus, because the efficiency of net photosynthesis increases, rate increases regardless of whether other factors limit gross photosynthetic rate (Long, 1991 and Long and Drake, 1992).

Therefore, the biochemical basis for the elevated atmospheric [CO₂] stimulation of C₃ photosynthesis is that high [CO₂] around Rubisco accelerates the carboxylation reaction while suppressing the competing oxygenation reaction and subsequently reducing the CO₂ loss and energy costs associated with photorespiration (Leakey *et al.*, 2009

and Long *et al.*, 2004). Changes in photosynthetic rates and acclimatory responses in C₃ plants grown under elevated CO₂ concentration could also be attributed to the feedback metabolic control wherein large accumulation of foliar starch and other carbohydrates could inhibit CO₂ assimilation rate whereas the plants with potential sinks for carbohydrate translocation and accumulation may not show any down-regulation of photosynthetic capacity suggesting that imbalances in source–sink could be attributed to the variations in the photosynthetic acclimation in different plants (Long *et al.*, 2004). Overall, limited sink strength (rate of CO₂ inflow towards the plant higher than its capacity to use assimilates in sink tissues) predisposes plants to a greater acclimation of photosynthetic capacity and decreases the stimulation of photosynthesis by growth at elevated [CO₂] (Long *et al.*, 2004 and Ainsworth and Rogers, 2007).

Sujatha *et al.* (2008) investigated how an increase in [CO₂] affects rice photosynthesis and carbohydrate metabolism. At elevated CO₂ (550 $\mu\text{mol mol}^{-1}$) concentration, the photosynthetic rates increased compared with plants grown at ambient CO₂. The photosynthetic rate, sucrose-P synthase (SPS) activity and accumulation of soluble sugars and starch were higher in the plants grown under elevated [CO₂]. They reported that the up-regulation of SPS (sucrose-P synthase) under elevated CO₂ would be beneficial for growth and productivity of rice plants for the future climatic conditions. The increased SPS activity was due to the reallocation of nitrogen away from Rubisco and into SPS, leading to a greater sucrose synthesis and export of photosynthates from the leaves. They concluded that rice responds to increasing CO₂ concentrations by shifting the priority of allocation of carbohydrates more towards export than carbohydrate storage, thus preventing the feedback inhibition.

2.4.2. Growth and yield responses under enriched [CO₂]

Growth and yield generally increased with exposure to elevated [CO₂], however, the magnitude of the response varied between species (Ainsworth and Long, 2005). C₃ photosynthesis is known to operate at less than optimal CO₂ levels and can show dramatic increase in carbon assimilation, growth and yields (DaMatta *et al.*, 2010).

Kimball (1983) did pioneering work in assessing the effect of elevated [CO₂] on crop and showed biomass increase of 10–143 per cent in several C₃ crops in response to

doubling of the ambient CO₂. Studies with rice indicated that elevated [CO₂] generally increases tiller number, photosynthesis, plant biomass and grain yield (Imai, 1995., Baker *et al.*, 1996., Kobayashi *et al.*, 1999 and Sakai *et al.*, 2001).

Upreti *et al.* (2000) investigated the growth response of rice (Pusa 834 variety) to increased CO₂ concentrations using open top chambers. CO₂ at crop canopy level was maintained between 575 to 620 ppm depending on outside wind velocity. Elevated CO₂ significantly increased the height of mother shoot and length of root to the tune of 16 per cent and 65 per cent over ambient grown plants, respectively. The dry weight of shoot was increased by 16 per cent while the increase in root dry weight was 200 per cent. The root-shoot ratio was markedly higher (155 per cent), production of leaves increased by 18 per cent, leaf area by 46 per cent, 113 per cent increase in dry weight of leaves compared to ambient grown plants. Elevated CO₂ significantly increased the grain yield by 53 per cent and seed number by 65 per cent and brought 10 to 15 per cent increase in the carbon content of leaves, stem and roots. Panicle number, filled grains per panicle and grain weight increased at high CO₂ but the general contribution of grain weight was less than the other two because of the negative correlation between grain number and grain weight. They concluded that future studies are needed to quantitatively analyse and to understand the physiological basis of the effect of elevated CO₂ climate on rice productivity (Upreti *et al.*, 2000).

Ainsworth, and Rogers (2007) reported that the increases in photosynthesis induced by high [CO₂] will need to be balanced by commensurate increases in sink capacity if maximum yield potential is to be achieved. Sujatha *et al.* (2008) reported that higher [CO₂] enhanced the activity of sucrose phosphate synthase and led to higher sugar production in rice crop. They indicated that additional sugars produced at the initial stages increased the number of productive tillers and this enhanced the production of panicles, which resulted in a higher number of grains. The higher amount of sugars produced at the heading stage was exported to these sinks, increasing the number of filled grains and grain mass, contributing thus to higher yield.

2.4.3. Role of Nitrogen in Photo acclimation

The alarming rise in the levels of atmospheric $[\text{CO}_2]$ and the coupled cycling of C and N is very critical to ecosystem function (Reich *et al.*, 2006). Nitrogen availability to plants, more than any other environmental factor, determines their responses to elevated $[\text{CO}_2]$ (Ellsworth *et al.*, 2004). This has been associated with the fact that elevated CO_2 results in decreased N content (typically by 13–16 per cent) in plant tissues (Curtis and Wang, 1998 and Ainsworth and Long, 2005). To maintain a balance in N and other resources allocated to the reactions that control photosynthesis, species acclimate to growth in elevated $[\text{CO}_2]$ (Drake *et al.*, 1997). This importance is highlighted by several kinds of studies suggesting that C-N interactions may substantially constrain the CO_2 fertilization effect at local and global scales (Hungate *et al.*, 2003 and Luo *et al.*, 2004). Photosynthetic acclimation (down-regulation) is frequently reported along with an accumulation of leaf nonstructural carbohydrates and a decrease in N concentration in the leaf and plant (Nowak *et al.*, 2004). Thus, nitrogen is required in relatively very large quantities for growth and development of plants, especially for plants grown under elevated CO_2 atmosphere (Reddy *et al.*, 2010).

Although the mechanisms by which elevated CO_2 decreases tissue concentrations of N (including proteins) are not yet fully understood, such decreases exceed what would be expected if a given amount of N were diluted by additional biomass (Bloom, 2006). The decreases in tissue N result to some extent from (i) the accumulation of carbohydrates and other organic compounds as a consequence of CO_2 stimulation of photosynthesis (Idso and Idso, 2001); (ii) reduced uptake (mass flow) of N from the soil under high CO_2 due to lower transpiration rates resulting from decreased stomatal conductance and changes in soil moisture status (Loladze, 2002 and Kimball and Bernacchi, 2006) and (iii) impaired nitrate assimilation associated with decreases in the photorespiration pathway at elevated CO_2 , as found in C_3 species such as wheat and tomato (Bloom, 2006). However, Makino and Mae (1999) reported that decreases in leaf N and rubisco contents are not due to dilution of N caused by a relative increase in the plant mass but as the result of a decrease in N allocation to leaves at the level of the whole plant and the decrease in Rubisco content is not selective. Makino *et al.* (1997) observed that CO_2 enrichment enhances the allocation of N to the leaf sheaths and roots. This means that

during long-term growth under elevated $[\text{CO}_2]$, plants reallocate N away from leaf blades to leaf sheaths and roots. In addition, since N invested into leaf blades is the most important source for photosynthesis in the whole plant, these results also indicate that plants regulate photosynthesis by changing N allocation at the whole plant level.

Decreases in the concentrations of Rubisco and other photosynthetic enzymes are found at elevated CO_2 without negatively compromising carbon acquisition (Drake *et al.*, 1997). However, decreased leaf protein may lead to reduced sink (e.g., grains and tubers) protein concentration as the N supply to sinks during filling is largely from translocation from catabolized proteins in senescing photosynthetic tissues (Hay and Porter, 2006). Therefore, at least on a theoretical basis, tissue nutrient concentrations and thus the nutritional value of crops are also expected to be affected under high CO_2 (DaMatta *et al.*, 2010).

2.4.3.1. Effect of nitrogen fertilization to rice under elevated CO_2

The C_3 plants often respond to CO_2 enrichment due to the fact that the carboxylation ability of rubisco commensurate with the enhanced CO_2 concentration in the atmosphere. However, such response may not reflect on the productivity due to the nitrogen deficiency. In order to address the response of elevated CO_2 in combination with N fertilization this thesis had an objective to assess the physiological responses of rice under elevated CO_2 regimes with graded levels of nitrogen. The literature pertaining to this aspect has been reviewed in this section.

Ziska *et al.* (1996) reported that neither above-ground nor below-ground biomass of rice responded positively to elevated $[\text{CO}_2]$ if there was no external N supplement, but at 90 and 120 kg N ha^{-1} , the stimulation of aboveground biomass and roots appeared to be proportional to N supply while roots demonstrated similar increases. The increase in aboveground biomass with elevated $[\text{CO}_2]$ was mainly attributed to increased photosynthesis and tiller number. For roots, it was attributable to the increased C allocation to the belowground, changes in root morphology as well as improved N use efficiency. In addition they also observed that microbial biomass in the surface 0–5 cm soil demonstrated significant increases under elevated $[\text{CO}_2]$, especially when N was not limiting plant growth.

Kim *et al.* (2001) assessed the effects of elevated CO_2 (300 $\mu\text{mol mol}^{-1}$ above ambient) on rice supplied with low, medium or high levels of N. For the medium and high N treatments, rice under elevated $[\text{CO}_2]$ levels increased N uptake at panicle

initiation but not at maturity. Highest total dry matter, spikelet number and grain yield were also observed in treatment comprising of elevated CO₂ and high N. These results indicated that to maximize rice grain yield under elevated CO₂, it is important to supply sufficient N over the whole season, in order to maintain the enhancement in dry matter production. In addition, N availability must be coordinated with the developmental stage of the crop, specifically to ensure that sufficient N is available at panicle initiation in order to maximize spikelet number and grain yield.

Anten *et al.* (2003) analysed the effects of elevated [CO₂] and nitrogen availability on canopy structure, leaf area index (LAI) and canopy photosynthesis of rice. Rice was grown at ambient and elevated (200 $\mu\text{mol mol}^{-1}$ above ambient using the free-air CO₂ enrichment, FACE) and at two N levels. FACE plants had higher light-saturated rates of photosynthesis and apparent quantum yields than ambient plants. Elevated [CO₂] reduced the total leaf N in the canopy but had no effect on LAI and the average leaf N content was therefore reduced by 8 per cent. They indicated that there is an interactive effect between nitrogen and carbon on canopy carbon gain. This interaction was caused by the increase in LAI with N availability, which enhanced the positive effect of the higher quantum yield under elevated [CO₂] (Anten *et al.*, 2003).

Li *et al.* (2004) investigated rice grown under 350 $\mu\text{L L}^{-1}$ (ambient) and 650 $\mu\text{L L}^{-1}$ (elevated) [CO₂] and different levels of nitrogen (N) supplement. Elevated [CO₂] demonstrated a wide range of enhancement to both above and below ground plant biomasses, in particular to stems and roots (for roots when N was not limiting) in the mid-season (80 days after transplanting) and stems/ears at the final harvest. In addition, elevated [CO₂] significantly increased microbial biomass carbon in the surface 5 cm soil when N (90 kg ha⁻¹) was in sufficient supply. Low N supplement (30 kg ha⁻¹) limited the enhancement of root growth under elevated [CO₂], leading consequently to diminished response of soil microbial biomass carbon. They concluded that the lack of understanding of the interaction between N nutrition and CO₂ enrichment is limiting further explanations for differing plant responses to elevated [CO₂] at varying levels of N supplement (Li *et al.*, 2004).

Weerakoon *et al.* (2005) observed that rice responds positively to increased atmospheric CO₂ concentration. However, the efficiency of the canopy depends on the N status of the plant, which could vary with the change in uptake and partitioning of N with increased atmospheric [CO₂]. Rice variety IR72 was grown inside open top chambers at ambient (350 µmol mol⁻¹) or elevated (700 µmol mol⁻¹) atmospheric [CO₂] in combination with three levels of applied N (0, 90 or 200 kg N ha⁻¹). When adequate N was not supplied, rice plants grown at high CO₂ became inferior to plants grown at ambient CO₂. When soil N supply diminishes, internal N recycling could trigger tissue death. In fact, tissue death in the N₀ treatment (control) was observed and total plant N under high [CO₂] was less than that of plants under ambient [CO₂]. N uptake and fertilizer N recovery was higher in plants grown in high [CO₂] until maximum tillering, but the partitioning of N towards leaves decreased by 9 per cent. They noted that acclimation to high [CO₂] by rice may, therefore, be dependent on the N uptake.

The overall literature review vividly suggests that carbon sequestration pattern in conventional and SRI cultivation of rice has been studied in seclusion and the data generated are quiet conflicting. However, the exhaustive literature review set a strong base to assess the relative performance of two distinct systems of rice cultivation in the perspectives of carbon sequestration and to evolve methane mitigation strategies.

Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

A series of experiments were conducted at the Department of Environmental Sciences, TNAU, Coimbatore to study the carbon sequestration pattern and methane mitigation potentials in SRI and conventional systems of rice cultivation, to verify the influence of moisture regime and nitrogen on soil carbon dynamics under controlled conditions and to assess the effect of elevated levels of CO₂ on carbon sequestration potential under Open Top Chambers (OTCs) conditions. The details of the materials used and methods followed during the course of investigation are described in this chapter.

3.1. Field experiment

3.1.1. Field location

Two field experiments were conducted during *summer* season (2011) and *kharif* season (2011) in 'D' block (field no. D5) and 'B' block (field no. B2) of the wetland farm, respectively in Tamil Nadu Agricultural University, Coimbatore. The farm is situated in Western Agro-climatic zone of Tamil Nadu at 11° North latitude and 77° East longitude with an altitude of 426.7 m above Mean Sea Level (MSL).

3.1.2. Climate and weather

The experimental location falls under semi-arid tropics and the normal weather condition of Coimbatore based on the mean weather data of 25 years indicated that the mean annual rainfall is 674 mm received in 47 rainy days. The mean maximum temperature and minimum temperature are 30.3 °C and 22.8 °C, respectively. The meteorological parameters such as temperature, relative humidity, rainfall wind speed, sunshine, solar radiation and evaporation recorded during the period of experimentation are furnished in Appendix 1.

3.1.3. Crop variety and seasons

A short duration rice hybrid 'CORH-3' was used in both *summer* (January-May) and *kharif* seasons (June - September). The seeds of CORH-3 were purchased from the Department of Rice, TNAU, Coimbatore. The parentage of the rice hybrid was TNAU

CMS 2A and CB 87R. CORH-3 has a yield potential of 7.5 t ha⁻¹ with duration of 115 days and attains flowering 80 days after sowing.

3.1.4. Design and Layout

The experiments were laid out in a split plot design with four replications. The layouts of the experimental field are presented in Fig.3.1 and 3.2.

Treatments:

Main plots

S₁ - Conventional method

S₂ - System of Rice Intensification (SRI)

Sub plots

N₀ - 0 kg N ha⁻¹

N₅₀ - 50 kg N ha⁻¹

N₁₀₀ - 100 kg N ha⁻¹

N₁₅₀ - 150 kg N ha⁻¹

Table 3.1. Details of important field operations

Details of operation	Summer (2011)	Kharif (2011)
Date of sowing	20.01.2011	24.06.2011
Date of transplanting for SRI	03.02.2011	08.07.2011
Date of transplanting for conventional	10.02.2011	15.07.2011
First conoweeding	13.02.2011	18.07.2011
Second conoweeding	23.02.2011	28.07.2011
Third conoweeding	06.03.2011	07.08.2011
Fourth conoweeding	16.03.2011	18.08.2011
Harvest	20.05.2011	24.10.2011

Fig. 3.1. Layout of the field experiment (Summer - 2011)

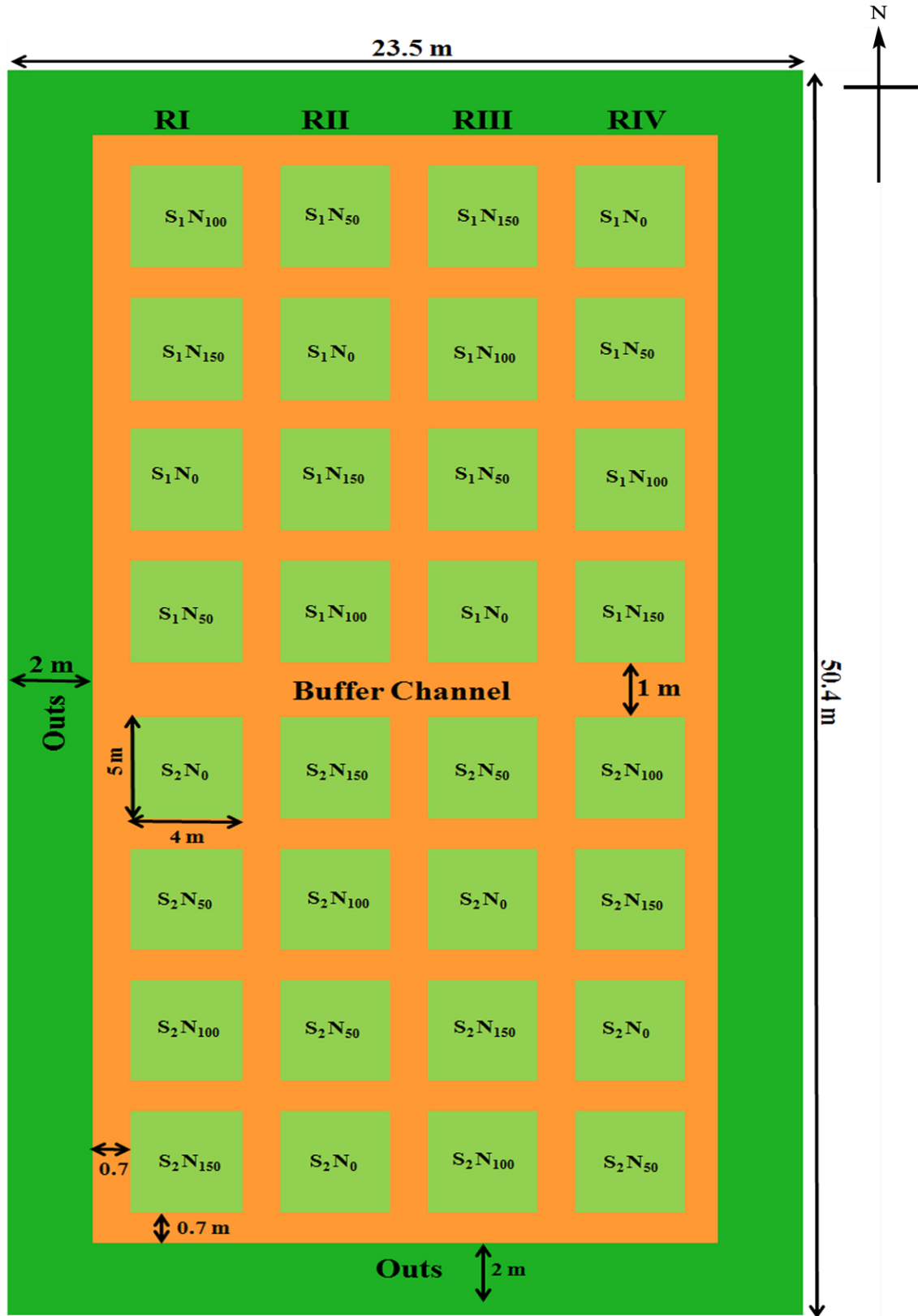
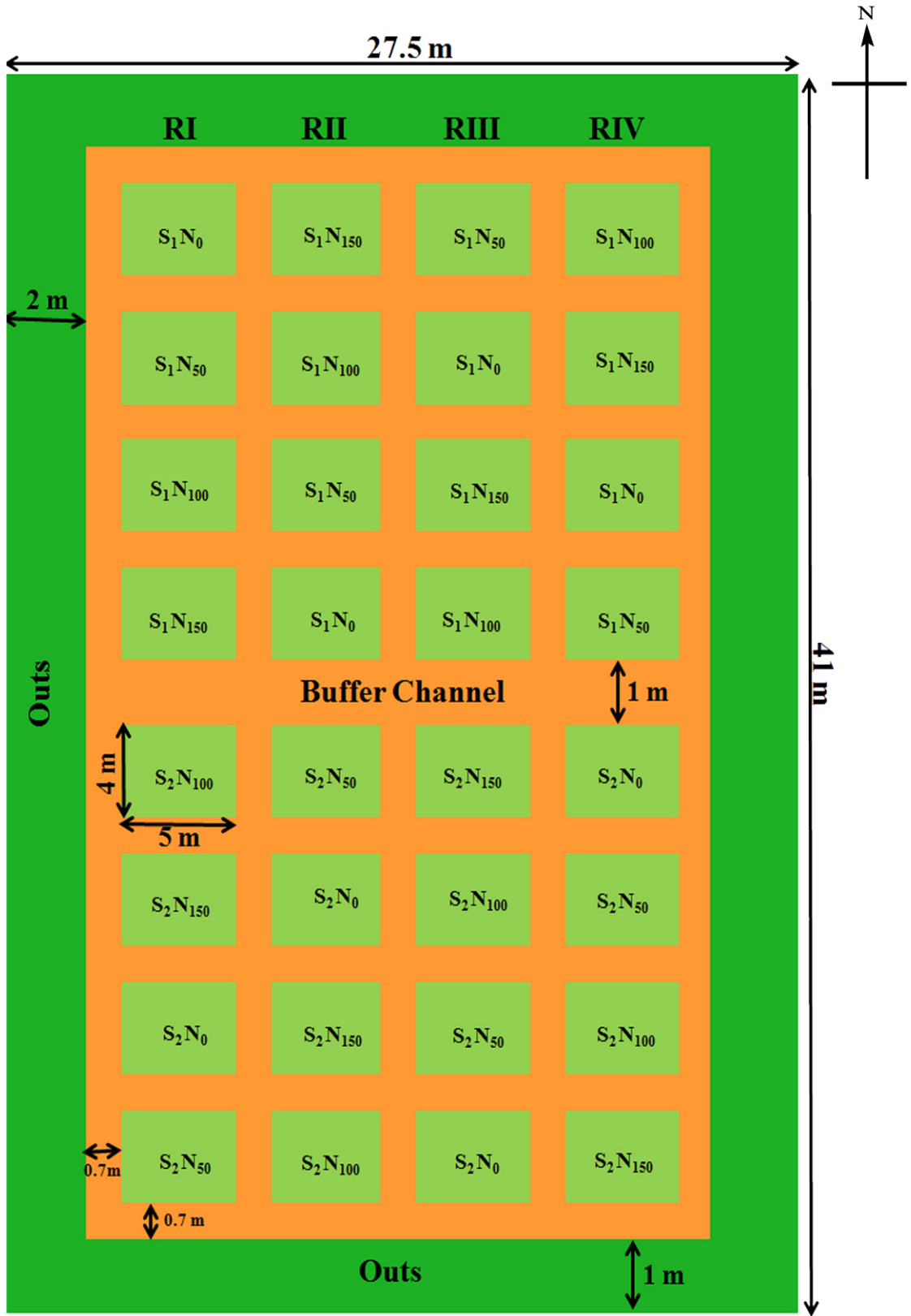


Fig. 3.2. Layout of the field experiment (*Kharif* - 2011)



3.1.5. Details of agronomic practices

3.1.5.1. System of Rice Intensification (SRI)

SRI is planting single young seedling in wider spacing and square pattern; using a weeder in between the hills; and adopting a water management strategy that keeps the soil moist. All the SRI practices were meticulously adopted as per the recommendations stipulated in the technical bulletin on SRI (Thiyagarajan *et al.*, 2009).

Seeds and sowing

In the present study, seed rate of 5 kg ha⁻¹ was followed as recommended for hybrids by the Department of Rice, TNAU, Coimbatore. The hybrid seeds (85 per cent germination) were sown after treating with *Pseudomonas fluorescences* (pf 1) at 10 g kg⁻¹ of seeds for raising nursery. This talc based formulation is mass multiplied in TNAU and recommended as a bio-control agent. After the treatment, the seeds were soaked in water for 12 hours and incubated in dark for 24 hours. The sprouted seeds were used for raising nursery on the same day.

Mat nursery

The nursery area was puddled before sowing and leveled well with wooden plank. In SRI, mat nursery with an area of 100 m² is recommended for planting one hectare. The mat nursery beds (5 cm height) were prepared on a polythene sheet base by mixing native soil, sand and FYM in the ratio of 4:1:1, respectively. The beds were prepared by scooping out the soil, so that furrows were formed all round the beds. Pre-germinated seeds were sown evenly and the furrows were irrigated to soak the beds. For subsequent irrigations, rose can was used to spray water. Initially, the beds were shaded with coconut fronds to maintain high humidity and facilitate uniform germination and establishment. At the time of transplanting, the nursery beds were taken in slices to the main field.

Main field preparation

The field was thoroughly puddled with power tiller and levelled with bullock drawn wooden plank. The field layout was prepared with buffer channels all round the plots (5m x 4m) to minimize the movement of nutrients and water. Since, good leveling

of main field is essential in SRI, final levelling was done manually to ensure uniform stand of water. A view of the experimental field is shown in Plate 1.

Inorganic fertilizer application

Recommended dosage of 150: 50: 60 kg ha⁻¹ N, P and K in the form of urea, single super phosphate and muriate of potash, respectively were applied. The N was applied in four splits at 10 DAT (50 kg ha⁻¹), 35 DAT (50 kg ha⁻¹), 55 DAT (25 kg ha⁻¹) and initial flowering stage (25 kg ha⁻¹). Phosphorus and zinc sulphate at the rate of 50 kg ha⁻¹ and 25 kg ha⁻¹ were applied basally before transplanting. The potassium was applied in four splits at the rate of 20, 20, 10 and 10 kg ha⁻¹, along with N fertilization.

Organic manure application

FYM obtained from the Department of Farm Management, Tamil Nadu Agricultural University was applied to the main field at the rate of 12.5 t ha⁻¹.

Transplanting

Fourteen days old seedlings from mat nursery were transplanted @ one seedling per hill in the main field at 25 x 25 cm spacing in the respective plots as per the layout plan. Care was taken to avoid transplantation shock. The seedlings were removed along with the soil intact with the roots and were transplanted immediately in such a way that the roots do not face upwards.

Gap filling

Gap filling in transplanted plots were carried out wherever necessary.

Water Management

The plots were irrigated only to moist the soil during the first 10 days. Then upto panicle initiation stage, field was irrigated to 2.5 cm depth after the previously irrigated water disappeared along with the development of hair line cracks. After panicle initiation, plots were irrigated to 2.5 cm depth one day after the previously ponded water disappeared from the surface.

Plate 1. A view of experimental field at Wetlands, TNAU



a. Summer



b. Kharif

Cono-weeding

Cono-weeder was used which resulted in incorporation of weeds with simultaneous stirring up of soil. It was operated in between the rows in both the directions (Plate 2). Cono-weeding was employed four times at an interval of ten days, starting from ten days after transplanting.

Plant protection

Need based plant protection measures were undertaken as per the recommendation for pests and diseases noticed in the experimental field.

Harvesting and threshing

The border rows in the plots were harvested first and the net plots were then harvested and threshed. Grain yield from net plot was brought to 14 per cent moisture by shade drying and expressed in kg ha^{-1} . The roots were separated from the plants and were washed with water, dried, weighed and expressed as kg ha^{-1} .

3.1.5.2. Conventional method

All the agronomic practices for conventional method were followed as per the standard recommendations stipulated in Crop Production Guide for Tamil Nadu (CPG, 2005).

Seeds and sowing

In the present study, the seed rate of 15 kg ha^{-1} was adopted since hybrid seeds were used. The hybrid seeds (85 per cent germination) were sown after treating with *Pseudomonas* at 10 g kg^{-1} of seeds for raising nursery. The seeds were soaked in water for 12 hours and incubated in dark for 24 hours. The sprouted seeds were used for raising nurseries on the same day.

Conventional nursery

In the present study, nursery @ 15 cents per ha was taken for raising hybrid seeds as recommended by the Department of Rice, TNAU. The nursery was puddled, leveled and the treated, sprouted seeds (15 kg ha^{-1}) were broadcasted uniformly with a thin film of standing water. The conventional nursery was managed well with irrigation and plant protection measures as per the recommendation.

Plate 2. Cono-weeding operations in SRI field



Main field preparation

The main field was prepared as mentioned in the previous section for SRI cultivation.

Inorganic fertilizer and FYM application

The quantity of inorganic and organic fertilizers and its schedule of application in conventional cultivation are similar to SRI.

Transplanting

Twenty one days old seedlings from conventional nursery were transplanted @ one seedling hill⁻¹ in the main field with the spacing of 20 x 10 cm in the respective plots as per the layout plan. In the present study, single seedling per hill was transplanted as recommended for hybrids by the Department of Rice, TNAU, Coimbatore.

Gap filling

Gap filling in transplanted plots were carried out wherever necessary.

Water Management

Water level of 2 cm was maintained up to seven days from the date of transplanting. After the establishment stage, cyclic submergence (5 cm) of water was continued throughout the crop period. Fields were irrigated one day after disappearance of ponded water.

Conventional weeding

Weeds were removed from the field manually in all the plots at 15 and 35 days after transplanting. The emulsifiable concentrate of butachlor @ 1.25 kg ha⁻¹ was applied as pre emergence herbicide by mixing with sand (50 kg ha⁻¹) at 3 DAT for transplanted crop with about 2 cm of standing water.

Plant protection

Need based plant protection measures were taken as per the recommendation for pests and diseases noticed in the experimental field.

Harvesting and threshing:

These operations were carried out as per the standard package of practices mentioned in the previous section 3.1.5.1.

3.2. Soil analysis**3.2.1. Collection of soil samples**

Representative soil samples from all plots were collected at active tillering, panicle initiation, flowering and at maturity stages of rice. The samples were air dried, powdered with a wooden mallet, sieved through 2 mm sieve and stored in polythene bags until further analyses.

3.3. Analytical methods**3.3.1. Mechanical analysis**

The mechanical composition of soil like coarse sand, fine sand, silt and clay were determined by the International pipette method (Piper, 1966).

3.3.2. Physical properties

Keen Raczkowski Box Method (Keen and Raczkowski, 1921) was adopted to determine the bulk density and particle density.

3.3.3. Chemical properties**3.3.3.1. pH and EC**

The pH of soil was measured in water (1:2.5) after half an hour equilibration with a glass electrode pH meter (Model: Elico LI 120). The electrical conductivity (EC) of the supernatant suspension was measured using a conductivity bridge (Model: Eutech PCD 5500) (Jackson, 1973).

3.3.3.2. Redox potential

Measurements for redox potential were done with each set of CH₄ flux measurement. The redox potential (Eh) of the field soil was measured by inserting a combined water proof ORP/ redox meter (Eutech Instruments, USA) to the soil and measuring the potential difference in mV (Satpathy, 1997). The Eh of soil was measured

(rhizosphere to bulk soil interface) in the morning and afternoon at different points near the flux measurement setup and averaged for the day.

3.3.3.3. Soil Organic Carbon

Soil organic carbon (SOC) was estimated by modified Walkley and Black (1934) method as described by Mebius (1960). Soil weighing 0.5 g (100 mesh sieved) was taken in a 500 ml conical flask and added 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 . The contents were digested with external heat of $140^\circ C$ for 30 minutes. Then distilled water (200 ml), H_3PO_4 (10 ml) and diphenylamine (1 ml) indicator were added. This was titrated against 0.5 N $Fe(NH_4)_2(SO_4) \cdot 6H_2O$ towards the end point of a bright green colour.

3.3.3.4. Soil Carbon Stock

The quantity of soil organic carbon stock in top 15 cm depth was calculated following the method described by Batjes (1996).

$$SOC \text{ stock (Mg ha}^{-1}\text{)} = \frac{SOC (\%) \times Bulk \text{ density (Mg m}^{-3}\text{)} \times 10,000 \text{ m}^2 \times \text{depth (m)}}{100}$$

3.3.3.5. Dissolved Organic Carbon (DOC)

Equilibrated soil solution samples were collected by zero tension sampling using soil water samplers (Tiensing *et al.*, 2001). The Dissolved organic carbon (DOC) was estimated by adopting the protocol of Nelson and Sommers (1996) with a slight modification as described by Lu *et al.* (2000). 2 mL of the soil solution was mixed with 3.0 mL of deionized water, 5.0 mL of 0.0175 M $K_2Cr_2O_7$, 10.0 mL of 98% H_2SO_4 , and 5.0 mL of 88% H_3PO_4 in a tube and digested for 30 min at $150^\circ C$. Upon cooling, the solutions were transferred to 150 mL Erlenmeyer flasks and titrated with 0.005 M $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 0.4 M H_2SO_4 solution and sucrose was used as a standard.

3.3.3.6. Active carbon pools

i. Soil microbial biomass carbon

Microbial Biomass Carbon (MBC) was determined by the fumigation-incubation technique (FIC) given by Jenkinson and Powlson (1976). Ten grams of soil sample was placed in 100 ml beakers and 5 ml of water was added to saturate the soil sample to 50 %.

The soil samples were placed in BOD incubator for 5-7 days at room temperature to trigger microbial activity. After the expiry of the incubation period, 2 mL of ethanol free chloroform was added to fumigate one of the two soil samples (fumigated) and the other beaker was kept as non-fumigated sample. Fumigated and non-fumigated soil samples were transferred to two beakers and sealed with wax paper to ensure elimination of atmospheric CO₂. The soil samples were incubated in room temperature for two days to evaporate the chloroform. Then the soils were transferred to conical flasks with a scintillation vial holding 5 ml of 0.5N NaOH (Fumigated samples) or 0.2N NaOH (non-fumigated samples) and the flasks were sealed and kept for 5 days. Evolved CO₂ was determined by titrating the alkaline traps with HCl (0.5 N) after precipitation of CO₃²⁻ with 50% BaCl₂ and using phenolphthalein as indicator. Biomass C was calculated from the net amount of (C fumigated- C nonfumigated) CO₂ – C evolved, using a K_C factor of 0.45 and multiplying CO₂ – C by it.

ii. Soil microbial biomass nitrogen

Microbial Biomass Nitrogen (MBN) was determined by the fumigation-incubation technique (FIC) described by Jenkinson and Powlson (1976). Ten grams of the soil was moistened to its 50 % water holding capacity. The moistened soil is fumigated or non-fumigated with ethanol free chloroform to eliminate and trigger microbial activities in the soil samples, respectively. The mineralizable in fumigated and non-fumigated soils is estimated using Bremner method. The soil samples are transferred to a Bremner apparatus and digested with mild alkali (MgO) and ammonia evolved is trapped in 2 % boric acid and the quantity of ammonia released is estimated as biomass nitrogen by using conversion factor of 0.54.

3.3.3.7. Passive carbon pools

Humic and fulvic acids were estimated by separation after extracting with 0.5 N NaOH. Their differential solubility in alkali and acid was adopted as the criterion for separating them into empirical forms (Stevenson, 1994).

i. Humic acid (HA)

Acid washed (0.1 N HCl) 40 g soil sample was taken in a polyethylene centrifuge bottle and 200 mL of 0.5 N NaOH was added. The mixture was shaken for 12 hours on a mechanical shaker and centrifuged at 3000 rpm for 10 min. Dark coloured supernatant liquid was filtered and the pH of the filtrate was adjusted to 1.0 with concentrated HCl. Additional 200 mL of 0.5 N NaOH was added to the residual soil, shaken, centrifuged and filtered. The residue was dispersed in 200 mL distilled water, centrifuged and the supernatant liquid was added to the previous extracts and the pH was adjusted to 1.0 with concentrated HCl and the humic acid was allowed to settle down. The supernatant liquid in the acidified extract was fulvic acid and it was siphoned off. The suspension was transferred to a polyethylene bottle and the HA was centrifuged off at 3000 rpm for 10 min. HA was redissolved in 0.5 N NaOH and re-precipitated with concentrated HCl. This purification was repeated several times. The supernatant liquid in each case was transferred to the original acid filtrate. HA was washed with distilled water until free of chloride. The HA extracted was dried in a rotary evaporator and ground to a fine powder. This was weighed and reported as percentage of organic matter.

ii. Fulvic acid (FA)

The acid extract collected in the HA preparation was FA. A known aliquot was taken, evaporated and dried. The residue was weighed and estimated as percentage of FA on moisture and ash free basis and finally expressed as percentage of organic matter.

iii. Non-humic organic fractions

The structural non-humus organic fraction was quantified by gravimetric method (Aiken *et al.*, 1985). A known quantity of oven dry soil was boiled with 0.1 N HCl (1:5 ratio) for 30 min to oxidize non-humic OM fraction. The suspension was decanted and the residue was washed with distilled water. Centrifugation was done to separate soil from the suspension. The soil residue was transferred to a porcelain dish and oven dried. The differential soil masses of pre and post acid treatment were used for deriving the non-humus soil organic matter fraction.

iv. Humin fraction

The humin fraction was estimated by deducting the cumulative mass of humic acid, fulvic acid and non-humus organic matter from the total soil organic matter content as suggested by Aiken *et al.* (1985).

v. Glomalin extraction and analysis

Glomalin from soil samples were extracted as described by Wright and Upadhyaya (1996). The total glomalin (TG) fraction was extracted with 50 mM sodium citrate (pH 8.0) at 121 °C for 60 min and then immediately centrifuged at 10,000 rpm for 5 min. After each cycle, the supernatant was removed and sodium citrate was replenished for the extraction of glomalin again until the supernatant showed no red brown color, which is typical for glomalin. The supernatant was stored at 4 °C for further analysis. Total glomalin fraction was analyzed using the Bradford protein assay using one mL of supernatant. Bovine serum albumin (BSA) was used as the standard for the assay. The glomalin concentrations in soils were expressed as mg g⁻¹.

3.3.3.8. Available nitrogen

The available N content of the soil was determined by the alkaline permanganate method (Subbiah and Asija, 1956). Five grams of soil (2 mm sieved) was taken in a distillation flask and 25 ml of each 0.32 % KMnO₄ and 2.5 % NaOH was added to the soil. Twenty ml of 2 % boric acid with a drop of double indicator was taken in a beaker and kept near the delivery end. The distillation was carried out and the liberated NH₃ was collected and titrated against 0.02 N sulphuric acid. From the titre value, the soil available nitrogen was calculated.

a. Mineral-Nitrogen

The fractionation of soil nitrogen was carried out by the procedure given by Cheng and Kurtz (1983). The individual fractions were estimated according to the procedure given by Bremner (1965).

b. Preparation of Equilibrium extract

A quantity of 10 g of soil (0.2 mm) treated with 100 ml of 2 M KCl solution was shaken for one hour and filtered through Whatman No.1 filterpaper.

i. Ammoniacal nitrogen (NH₄-N)

An aliquot of 20 ml of the above filtrate was distilled with freshly ignited MgO in distillation apparatus and the distillate collected in 2 % boric acid containing mixed indicator was titrated with standard H₂SO₄.

ii. Nitrate nitrogen (NO₃-N)

On removal of NH₄ from the sample, a pinch of Devarda's alloy was added and continued the steam distillation. The amount of NO₃-N was determined as described for NH₄-N.

3.3.3.9. Available Phosphorus

Soil available phosphorus was determined by Olsen's extractant method (Olsen *et al.*, 1954). Five gram of soil was taken in a polycarbonate shaking bottle. Fifty ml of 0.5 M NaHCO₃ adjusted to pH 8.5 and a pinch of activated carbon (Dargo G 60) were added to the soil and shaken for 30 min. The extract was filtered using Whatman No. 40 filter paper. Five ml of filtrate was pipetted out into a 25 ml volumetric flask and 4 ml of reagent B was added and made up to 25 ml. After 30 min. the absorbance value of the colour developed in the sample was read at 660 nm in a spectrophotometer (Elico-Model BL 198) and the available phosphorus was calculated from the standard curve.

3.3.3.10. Available Potassium

The available K as extracted in neutral normal NH₄OAc was determined. Five grams of the soil was taken in a 100 ml shaking bottle and 25 ml of 1 N NH₄OAc was added, shaken for 5 min. and then filtered. The NH₄OAc – K in the extract was determined using a flame photometer (ESICO – Model: 1382) (Jackson, 1973).

3.3.4. Biological properties**a. Soil microbial enumeration**

The population density of bacteria, fungi and actinomycetes were enumerated using serial dilution plate technique (Parkinson *et al.*, 1971).

b. Serial Dilution of the sample

One g of the air dried soil was transferred to 10 mL of sterile distilled water to get 10^{-1} dilution. After thorough shaking one mL of the dilution was transferred to 9 mL water blank to get 10^{-2} dilution. Likewise, sample was diluted serially with 9 mL water blanks until appropriate dilution was obtained.

3.3.4.1. Bacteria

The bacterial population was enumerated by plating one mL of 10^{-6} dilution in sterile Petri dishes using nutrient agar medium. The bacterial colonies appearing on the plates after 48 hours of incubation at 30°C were counted and expressed as CFU g^{-1} of dry soil.

3.3.4.2. Fungi

The fungal population was enumerated by plating one mL of 10^{-4} dilution in sterile Petri dishes using Martin's rose bengal agar medium. The fungal colonies appearing on the plates after incubation for 3-5 days were counted and expressed as CFU g^{-1} of dry soil.

3.3.4.3. Actinomycetes

The population of actinomycetes was enumerated by plating one mL of 10^{-3} dilution in sterile Petri dishes using Kenknight's agar medium. The actinomycetes colonies appearing on the plates after incubation for 7 days were counted and expressed as CFU g^{-1} of dry soil. The composition of the growth medium used above is given in the Appendix 2.

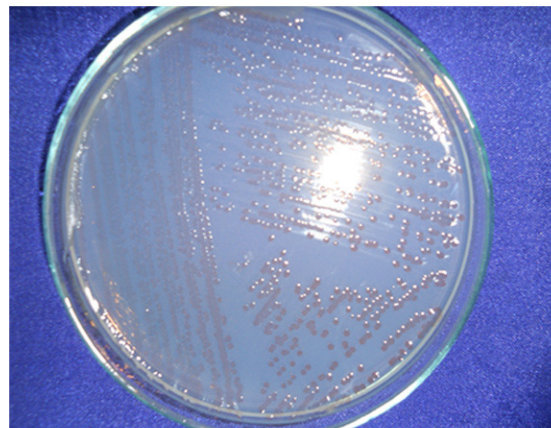
3.3.4.4. Methanotrophs

Nine ml of phosphate buffer solution were taken in test tubes representing upto 10^{-6} dilution. Then the tubes were sterilized in autoclave at 15psi for 20 minutes. One gram of soil sample was taken and serially diluted. Dilutions of 10^{-3} were used for plating. Pour plate technique was performed using Noble Agar Medium (Appendix 3) and the plates were incubated in the Macintosh jar assembly (Plate 4) with provisions for attaching a bladder containing methane. Methane was provided as the carbon source for the growth of methanotrophs in the chamber and the plates were incubated for 7 to 15 days. Methane gas was replenished once in two days through the bladder. After fifteen days of incubation, the plates were removed and o-dianisidine dye test was performed for assessing the

Plate 3. Air sampling using static closed chamber



Plate 4. McIntosh jar assembly and colonies of methanotrophs



methanotrophs activities in the presence of naphthalene crystals. Few naphthalene crystals were sprinkled on the plate lid and stored inverted for 15 minutes. Then the plates were opened and freshly prepared o-dianisidine (tetrazotized; zinc chloride) was sprayed and incubated for 15 minutes in the presence of the dye. Methanotrophs exhibit methane mono oxygenase (MMO) activity and hence, naphthol was produced and purple red colour colonies were observed. Methanotrophs colonies (Purple red) colonies were counted and expressed as CFU g⁻¹ of dry soil. The methanotrophic bacteria was isolated and presented in the plate 4.

3.3.4.5. Enumeration of anaerobic microflora from rhizosphere

i. Collection of soil samples

Soil samples were collected at active tillering, panicle initiation, flowering and harvest stages and enumerated for the population of anaerobic micro-flora. The samples were collected at the lower horizon (10 cm depth) under anaerobic conditions (Ramasamy *et al.*, 1992).

ii. Preparation of dilution medium

The dilution medium consisting of 0.5% sodium bicarbonate, 1.0% sodium carbonate and 0.001% resazurin was prepared and boiled under CO₂ atmosphere. To 100 ml serum vial, 90 ml of the cysteine HCl reduced dilution medium was added, stoppered and autoclaved at 15 lbs pressure for 20 min. Soil samples collected anaerobically were transferred to the above vials. The soil samples were further diluted by using the dilution medium and appropriate dilutions were used for the enumeration of total anaerobes (10⁶), 10⁴ for methanogens and 10² sulphate reducers.

iii. Enumeration by roll tube technique

The appropriate media listed in the appendix 4, were prepared and sterilized. Anaerobes were enumerated by adopting the roll tube technique (Hungate, 1957). The sterilized medium was placed in a water bath at 45-50°C to maintain the liquid state while it was flushed continuously with CO₂/N₂ using the gassing manifold assembly. Simultaneously the sterile test tubes were kept under CO₂/N₂atmosphere. The samples were also maintained under CO₂/N₂ atmosphere. One ml of trace element solution and

vitamin solution was added to the media before transferring to the sample. One ml of the sample was transferred from the desired dilution to the sterile test tube of 25 ml capacity which again was kept under CO₂/N₂ atmosphere. By using a sterile pipette (10 ml capacity) with silicon tube at one end, 5 ml of the medium was transferred to the test tube with sample which was also maintained under CO₂ atmosphere. The test tube was immediately stoppered with sterile rubber corks by simultaneously drawing out the gassing jet. The test tube was rolled over the foam, soaked in cold water till the medium uniformly solidified on the sides of the test tube. The test tubes were incubated in an anaerobic jar.

iv. Total anaerobes

The population of total anaerobic bacteria was enumerated using Hungate's medium (Hungate, 1957) and roll tube technique. After the incubation period the number of colonies in the roll was counted and recorded.

v. Methanogens

The population of methanogens was estimated by using Mah's medium (Mah, 1980). The colonies were identified by their bluish florescence under UV light.

vi. Sulphate reducers

Sulphate reducers were enumerated by isolating the black colonies that appeared on Postgate's medium (Postgate, 1981).

3.3.4.6. Enzymatic activities

a. Dehydrogenase activity

Soil dehydrogenase activity was determined by the method outlined by Casida *et al.* (1964). Five grams of soil sample was taken in polybottles with a reaction mixture containing 2.5 ml distilled water, 1 ml of 3 % solution of 2,3,5– triphenyl tetrazolium chloride, 2.5 ml of 0.1 M phosphate buffer of pH 7.1. The contents of each tube were then mixed thoroughly at 25°C for 24 hours. Then 10 ml of methanol was added and incubated for another 5 hrs. The content was filtered through Whatman No.1 filter paper. The sample was washed thoroughly with methanol. The brick red colour developed was read

at 485 nm in a spectrophotometer (Elico BL 198). The concentration of dehydrogenase in the sample was obtained from the standard graph using triphenyl formazone.

Phosphatase activity

Five grams of soil sample was taken in boiling tube. To this 10 mL of distilled water, 0.25 mL of toluene and 1 mL of 10 mM para nitro phenyl phosphate (pNP) were added and incubated at room temperature for 1 hour. Then 5 mL of 0.5 M CaCl_2 and 20 mL of 0.5 M NaOH were added. The content was filtered using Whatman No 42 and volume was made up to 50 mL with distilled water. The intensity of yellow colour developed was read at 420 nm in spectrophotometer (Elico BL 198). The activity of phosphatase was calculated using standard graph (Bremner and Tabatabai, 1969).

c. Urease activity

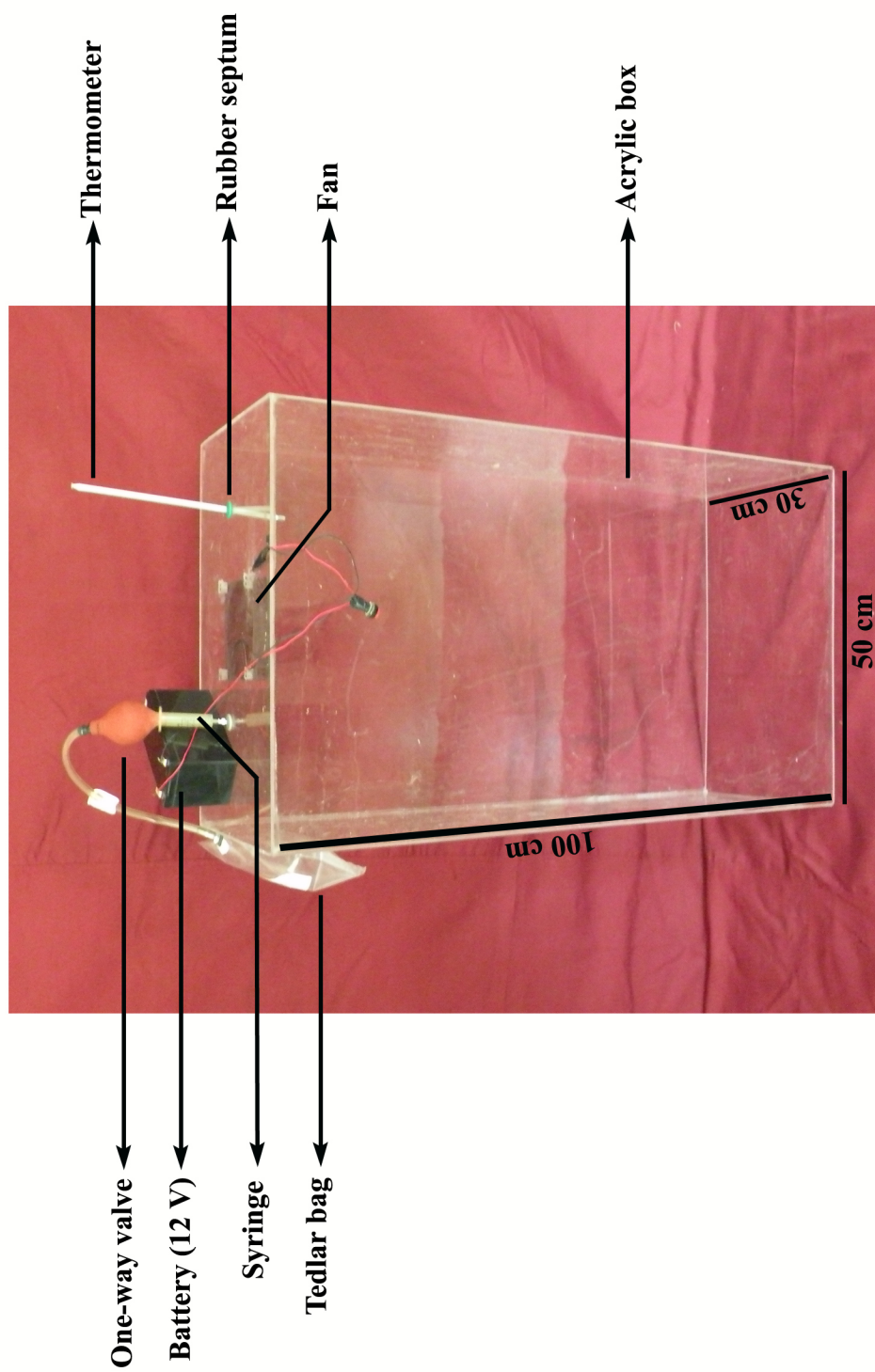
To one gram of moist soil, 1 mL of 3% urea and 2 mL of 0.1 M phosphate buffer (pH 7.1) were added and kept in water bath at 37°C for 1 hour. The ammonia formed during incubation was extracted with 50 mL of 2 M KCl. 10 mL of extract was used for distillation with 0.2 g ignited MgO. The ammonia evolved was trapped in 2% boric acid and was titrated against 0.02 N sulphuric acid. Similarly, duplicates were maintained without the addition of 3% urea (Hofmann and Teicher, 1961). Urease activity was obtained from the difference in ammonia formed and expressed as $\text{mg of NH}_4\text{-N released g}^{-1}\text{soil hr}^{-1}$.

3.4. Quantification of CH_4 and CO_2 emissions

3.4.1. Fabrication of Closed Chambers

Gas samples were collected from the field using static closed chamber technique (Minami and Yagi, 1988; Jain *et al.*, 1999). The gas chambers were fabricated as per the recommendations of several studies on trace gas measurements in field conditions (Mosier, 1989; IAEA, 1992; Adhya *et al.*, 1994; Denmead, 2008). The closed chamber used in the present study is presented in Fig 3.3. Open-bottom perspex chambers made of 4 mm acrylic sheets with a dimension of 50cm x 30 cm x 100 cm were fabricated. A battery (12V) operated fan was fixed inside the chamber to homogenize the inside air before collection of air samples. The lid of the chamber was fitted with terumo venoject rubber

Fig. 3.3. Static closed chamber



inserts to permit the introduction of syringes and suspension of NaOH vials inside the chamber. A thermometer is also fitted to monitor the inside temperature.

3.4.2. Collection of air samples for CH₄ and CO₂ estimation

As described by Khosa *et al.* (2010) metal frame was fixed into the soil one hour before air sampling and the perspex chamber was kept over it at the commencement of the measurement (zero time) so as to minimize the disturbance to the soil and water. After mounting the chambers on the metal frame, water was added into the grooves in order to make the system air-tight. Care was taken not to disturb the vegetation during the whole measurement programme. After covering the plants with the chamber, four air samples were collected in Tedlar bags (DuPont, AeroVironment, California) starting with zero time and subsequent sampling at an interval of 15 minutes using syringe and one-way valve pump (Plate 3). As described by Rath *et al.* (1999) and Jayadeva *et al.* (2009), the air samples were collected in the morning (09:00-10:00 hours) and in the evening (14:00-15:00 hours) and the average of morning and evening fluxes were used as the flux value for the day. Soil temperature, air temperature and soil redox potential was also simultaneously recorded at the time of air sampling.

Gas samples were collected continuously for a week at critical stages of crop growth viz., active tillering, panicle Initiation, flowering and maturity stages and the average of the seven days were reported as the average daily methane and carbon-dioxide emission rate for the respective stage. In order to study the effect of cono-weeding on methane emissions, air samples were also collected before and after every cono-weeding operation during the *kharif* season (2011).

3.4.3. Estimation of CH₄ and CO₂ flux

The air samples collected in tedlar bags were brought to the fermentation laboratory at the Department of Environmental Sciences, TNAU and analysed using gas chromatograph (Varian – CP 3800). Methane was estimated using GC with a flame ionization detector (FID). The temperature for the column, injector and detector was kept at 50°C, 75°C and 250°C, respectively. Methane was separated from other gaseous components on a Porapak N column (80-100 mesh, 2 m long stainless steel). The repeatability of the system was better than $\pm 2\%$. All the samples were analysed against a laboratory

reference which had been calibrated using a known mixture of pure methane with an error of about 1-2%. The GC was calibrated before and after each set of measurements using 95 per cent pure CH₄ obtained from Chemtron Sciences Laboratories Pvt. Ltd., Mumbai and used as primary standard. Carbon-dioxide concentration was analysed using GC equipped with thermal conductivity detector. The column temperature was set at 150°C and injector and detector temperatures were set at 200°C. GC was calibrated using 5 ppm CO₂ obtained from Chemtron Ltd. CH₄ and CO₂ concentrations were determined by peak area and flux was calculated based the equation proposed by Rolston (1986) to estimate methane and carbon dioxide emissions.

$$f = (V/A) (\Delta C / \Delta t)$$

where f is equal to greenhouse gas emission rate ($\text{mg m}^{-2} \text{h}^{-1}$), V is equal to volume of chamber above soil (m^3), A equal to cross-section of chamber (m^2), ΔC equal to concentration difference between zero and t times (mg cm^{-3}), and Δt equal to time duration between two sampling periods (h). The air samples were also analyzed using another gas chromatograph (Shimadzu GC-2014) for comparison. The values obtained by two chromatographs did not differ by more than 4 %. The total methane and carbon-dioxide flux for respective seasons were estimated by multiplying the averaged flux value of the particular stage with its duration.

3.5. Biometric observations

The following biometric observations were recorded at important physiological crop growth stages viz., active tillering, panicle initiation, flowering and maturity by adopting standard procedures.

3.5.1. Growth attributes

3.5.1.1. Plant height

The plant height was recorded at active tillering, panicle initiation, flowering and maturity stages and expressed in cm

3.5.1.2. Leaf Area Index (LAI)

The LAI of rice was worked out using the formula outlined by Palanisamy and Gomez (1974)

$$\text{LAI} = \frac{L \times W \times K \times \text{Number of leaves per hill}}{\text{Area occupied by the plant (cm}^2\text{)}}$$

where,

L = Maximum length of 3rd leaf blade from the top (cm)

W = Maximum width of the same leaf (cm)

K = Constant Factor

3.5.1.3. Number of tillers per hill

Five hills were selected at random in the net plot area and the number of tillers hill⁻¹ was recorded.

3.5.1.4. Root volume

Root volume was measured using water displacement method and expressed in cc plant⁻¹.

3.5.1.5. Root biomass

After measuring the root volume, the roots were dried in shade and then oven dried at 70°C to constant weight and the dry weight was recorded in g hill⁻¹ and also expressed as kg ha⁻¹.

3.5.2. Yield components

3.5.2.1. Productive tillers

Five hills were selected at random in the net plot area and the productive tillers hill⁻¹ was recorded. In addition, 1m x 1m quadrat was employed to count number of productive tillers and expressed as productive tillers per m⁻².

3.5.2.2. Grain and straw yields

Grain and straw were harvested in each plot leaving two border rows on all sides. The harvested produce from each plot was threshed manually and sun dried, winnowed separately and weighed. Moisture content was estimated and the grain yield was adjusted

to 14 per cent moisture content as suggested by Yoshida and Paraoq (1976) and expressed in kg ha^{-1} . Straw from each plot was sun dried and yield was recorded and expressed in kg ha^{-1} .

3.5.2.3. Harvest Index

The harvest index was calculated by using the following formula suggested by Donald and Humblin (1976)

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield (grain yield + straw yield)}}$$

3.6. Plant analysis

The plant samples were cleaned with water and separated into grains, roots and straw. Plant samples were shade dried and later oven dried at 70°C . After recording the dry weight, each sample was ground in a Willey mill and sub-samples were obtained for laboratory analysis.

3.6.1. Carbon content

The carbon content of straw, root and grains were measured by using high temperature TOC analyser (Elementer, Model: Liqui TOC II). Temperature was maintained at 850°C with a pressure of 0.9 to 1 bar. Pure oxygen was used as a synthetic gas.

3.6.2. Nitrogen content

The nitrogen content of the straw, root and grains were determined by the procedure described by Humphries (1956). About 10 mL of the diacid (H_2SO_4 and HClO_4 in 5:2) digest was taken and transferred to microkjeldahl to distill the ammonia. 2% boric acid with 2-3 drops of double indicator was used to collect evolved ammonia and titrated against 0.02 N sulphuric acid. Blanks were maintained without adding sample.

3.7. Incubation experiment

Bulk soil samples were collected from wetland farm of Tamil Nadu Agricultural University, Coimbatore. Soils were shade dried, sieved (2 mm) and characterized for initial soil organic carbon content. One kilogram capacity jars loaded with seven hundred grams of soil were used for the incubation study to assess the organic carbon dynamics

and CO₂ evolution. The treatment comprised of two levels of nitrogen viz., control and nitrogen @ 0.0075% (w/w basis). The soils were subjected to two different moisture regime viz., i) alternate wetting and drying and ii) submerged (flooded) conditions. The experimental design adopted was factorial completely randomized design with five replications. CO₂ liberated was estimated using alkali trap method as described by Stotzky (1965). The CO₂ trapped in 0.5 M NaOH was determined by titration with 0.1M HCl after adding excess of 3 M BaCl₂ as per the following equation (Stotzky, 1965) and CO₂ evolved was expressed as mg kg⁻¹ of soil. Soil samples were also collected at every seven days interval and analysed for soil organic carbon content.

$$\text{Milligrams of C (or) CO}_2 = (B-V) NE$$

Where,

B = Volume (ml) of acid needed to titrate the NaOH in the vials from control flasks.

V = Volume (ml) of acid needed to titrate the NaOH in the vials from flasks of different treatments.

N = Normality of acid.

E = Equivalent weight

(To express in terms of C, E=6; to express it as CO₂, E=22)

3.8. Response of rice to elevated CO₂

It is estimated that the year 2050 and 2100 are the critical time frame for evaluation where the atmospheric CO₂ concentrations is likely to exceed 550 and 750 ppm, respectively (IPCC, 2007). Therefore, the effect of elevated levels of CO₂ viz., 550 μ mol mol⁻¹ CO₂ and 750 μ mol mol⁻¹ CO₂ on rice crops were investigated by employing Open Top Chambers installed at the Department of Soil Science & Agricultural Chemistry, TNAU, Coimbatore.

3.8.1. Description of Open Top Chambers (OTCs)

The OTCs are of square type structure with a dimension of 3x3x3 m (Plate 5). The basic structure of the OTCs were fabricated with galvanized iron (GI) pipes and covered with polyvinyl chloride (PVC) sheets of 120 micron gauge to ensure more than

Plate 5. Open Top Chamber (OTC) facility at TNAU



90 per cent transmittance of light. At 2.4 m height of the each chamber, a frustum with an angle, 0.6 m towards inside was maintained to reduce the dilution effect of the air current within the chamber. The upper portion of the chamber was kept open to maintain the near-natural conditions of the temperature and relative humidity. Through the vertical vent pipes, CO₂ is released towards the centre of the chambers. The 100 per cent CO₂ gas of commercial grade was used to elevate CO₂ levels within the chambers. CO₂ gas cylinders of 45 kg capacity were used and the gas was released to the chambers through a manifold fitted with copper tubing. Within each chamber, the copper tubing was again fitted with solenoid valve and rotameters to regulate the gas supply. The normal air from the air compressor was mixed with CO₂, which is then pumped into the CO₂ circulating pipe. The CO₂ was released into the OTCs through perforated GI pipes. The uniformity of the CO₂ was maintained by pumping CO₂ gas diluted with air by air compressor. Sensors for CO₂, relative humidity, soil temperature, air temperature and solar radiation were installed inside as well as outside the chambers. The maximum and minimum air temperature variations in OTCs and ambient conditions were furnished in Annexure 5.

The equipment for monitoring and controlling the CO₂ in OTCs was fully automated and desired CO₂ concentrations (550 ppm and 750 ppm) were maintained throughout the experimental period. The entire system was linked to data logger and computer system with UPS for uninterrupted data recording and storing.

3.8.2. Pot experiment

Seven kilograms of wetland soil (air dried, 2mm) was weighed and transferred into a syntex pot (top diameter 18 cm, bottom diameter 16 cm, height 20 cm and the holes at the bottom were sealed for the purpose of water stagnation). The FYM at the recommended dose of 12.5 t ha⁻¹ (41.6 g pot⁻¹) and NPK at 150:50:60 kg ha⁻¹ (500, 166.6, 200 mg NPK pot⁻¹) were applied in the form of urea, single super phosphate and muriate of potash, respectively. Zinc sulphate at rate of 25 kg ha⁻¹ (83.3 mg pot⁻¹) was applied and was thoroughly mixed with the soil. The N and K was applied in four splits and P was applied basally before transplanting. Rice crop was treated with three different levels of nitrogen viz., 0, 150, 200 kg N ha⁻¹ and the N was applied in four splits on soil weight basis.

Pot culture experiment was conducted during summer season (2012) and the seeds were sown on 10.02.2012. Nursery was raised in the wetland farm and 14 days old paddy (ADT 45) seedlings were transplanted into the pots. After establishment, two healthy seedlings were allowed to grow in each pot. Twenty five days old rice crop was subjected to different CO₂ atmospheric conditions (380, 550 and 750 ppm $\mu\text{mol mol}^{-1}\text{CO}_2$) and the rice crop was continuously kept under these conditions until harvest. The pots were maintained under flooded conditions (cyclic submergence) throughout the crop period. The plants were harvested on 05.06.2012. A view of rice plants exposed to elevated [CO₂] is shown in Plate 6.

Treatments

Design : FCRD

Replications : Five

Factor 1:

C_{amb} - Ambient CO₂ concentration (380 $\mu\text{mol mol}^{-1}\text{CO}_2$)

C₅₅₀ - 550 $\mu\text{mol mol}^{-1}\text{CO}_2$

C₇₅₀ - 750 $\mu\text{mol mol}^{-1}\text{CO}_2$

Factor 2:

N₀ - 0 kg N ha⁻¹

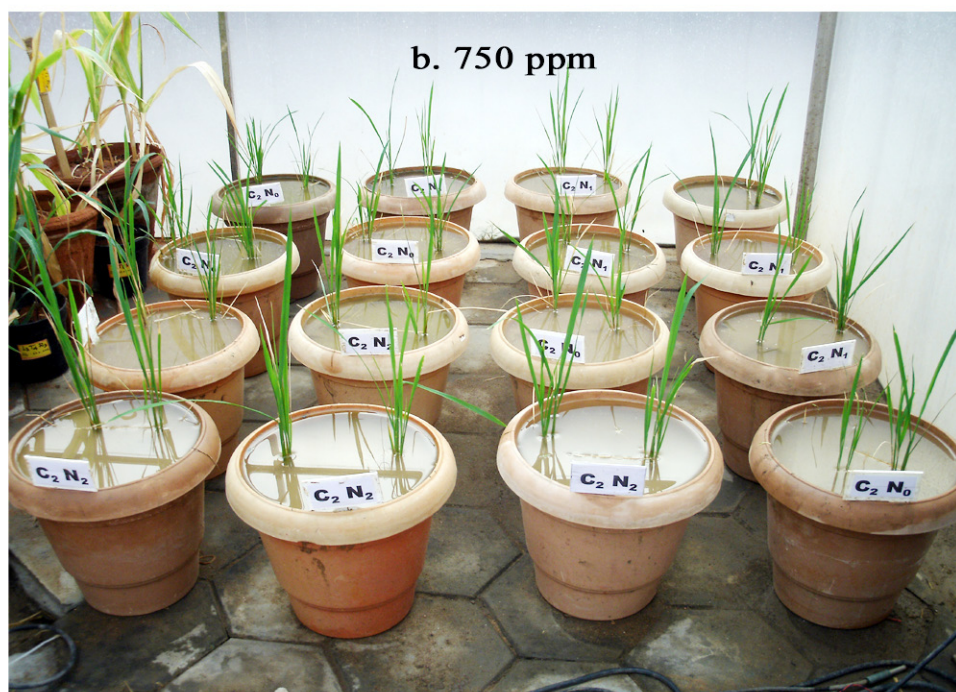
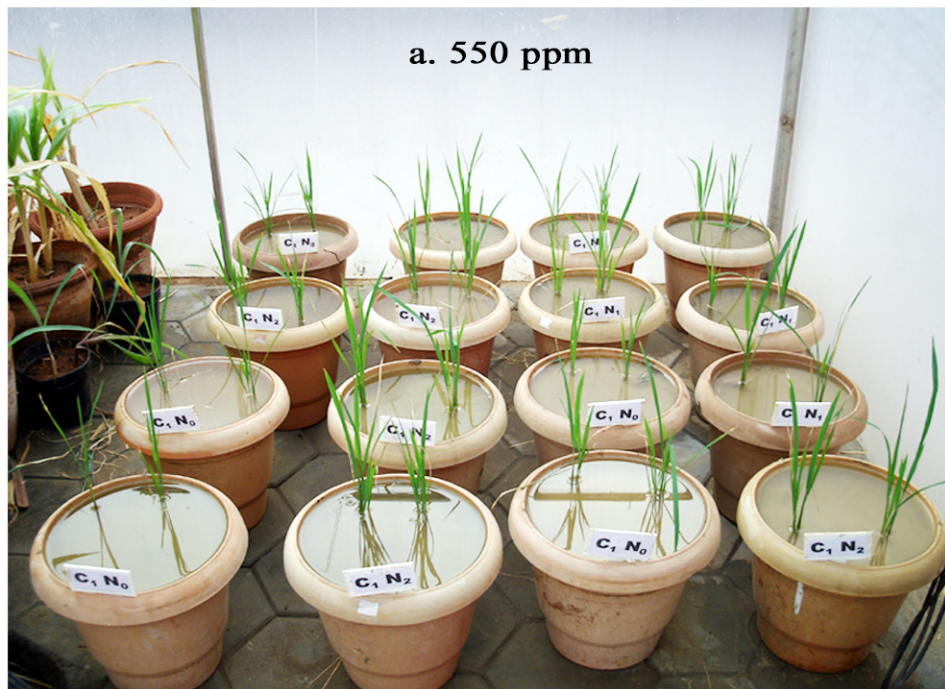
N₁₅₀ - 150 kg N ha⁻¹

N₂₀₀ - 200 kg N ha⁻¹

3.8.3. Soil analysis

Soil samples from all the pots were collected at active tillering, flowering and maturity stages of rice. The samples were air dried, powdered with a wooden mallet, sieved through 2 mm sieve and stored in polythene bags until further analyses. The parameters such as soil organic carbon, dissolved organic carbon, microbial biomass carbon, microbial biomass nitrogen, available macro-nutrients, population of methanogens and methanotrophs were analyzed as per the standard analytical methods outlined in the previous section.

Plate 6 . A view of rice plants exposed to elevated $[\text{CO}_2]$ in OTC



3.8.4. Estimation of methane flux

Gas samples were collected from the pots using static closed chamber technique and the gas chambers (250 mm diameter and 890 mm height) were fabricated in such a way that it fits the pot. The other specifications and components are similar to the chambers used for field measurements. Gas samples were collected at active tillering, flowering and harvest using tedlar bags and the protocol followed for collection and estimation were carried out as per the standard procedure mentioned in the previous section 3.1.6.2.

3.8.5. Biometric observations

The biometric observations such as plant height, number of tillers per hill were recorded at crop growth stages viz., active tillering, flowering and maturity. The yield attributes such as productive tillers per hill, days to 50 % flowering, number of grains per panicle and number of filled grains per panicle, 1000 grain weight, grain yield, straw yield and root yield are recorded by adopting standard procedures.

3.8.6. Physiological parameters

At each growth stage of rice, the following parameters were analyzed.

3.8.6.1. Chlorophyll content

Fresh leaf samples (250 mg) were macerated in a pestle and mortar with 10 ml of 80 per cent acetone and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the volume was made up to 25 ml using 80 per cent acetone and the chlorophyll content was obtained by measuring the OD at 645, 663 and 652 nm in spectrophotometer (Elico BL 198) and expressed in mg g^{-1} fresh weight (Bruinsma, 1963).

3.8.6.2. Soluble proteins

Soluble proteins in rice leaves were determined by the Folin phenol method (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a standard. 250 mg of root or leaf tissue were macerated with 10 mL 0.2 M phosphate buffer and centrifuged at 3000 rpm for 10 min. One mL of supernatant solution was mixed with 5 mL alkaline copper tartarate reagent (2% Na_2CO_3 in 0.1 N NaOH and 0.5% CuSO_4 in 1% sodium potassium tartarate mixed in 50:1) and kept for 30 min for the biuret reaction to take place. Soluble proteins

content was estimated by measuring the absorbance of blue colour that developed with Folin Ciocalteu reagent (1 part of Folin Ciocalteu reagent mixed with 2 parts of distilled water) at 660 nm in spectrophotometer (Elico-Model BL 198). The soluble protein content was expressed as mg g^{-1} .

3.8.6.3. Nitrate Reductase (NRase)

Nitrate reductase activity in leaves and roots were determined by adopting the method of Nicholas *et al.* (1976). One gram of fresh tissue was homogenized in the medium containing 1mM EDTA, 1-25 mM cysteine and 25 mM potassium phosphate adjusted to a final pH 8.8 with KOH. Filtration was carried out using cheese cloth and then centrifuged for 15 min at 30,000 g. The reaction was initiated by addition of 0.2 ml enzyme extract in to the test tube containing 0.5 ml phosphate buffer, 0.2 ml potassium nitrate, 0.4 ml NADH solution and 0.7 ml of water. After incubation for 15 min at 30°C, the reaction was terminated by the addition of 1 ml sulphanilamide followed by 1 ml naphthyl ethylenediamine reagent. Nitrate reductase activity was measured at 540 nm after 90 minutes. The enzyme activity was expressed as $\mu\text{mole NO}_2 \text{ hr}^{-1} \text{ g}^{-1}$ fresh weight.

3.8.6.4. Glutamine Synthetase (GS)

Glutamine synthetase activity in leaves and roots were determined by adopting the procedure described by Sadasivam and Manickam (1991). Enzyme extract was prepared by taking one gram of leaves and roots in 5 ml of 50 mM imidazole-acetate buffer pH 7.8 containing 0.5 mM EDTA, 1mM dithiothreitol, 2 mM MnCl_2 and 20 per cent glycerol centrifuged at 10,000 g for 30 min. The supernatant was collected and used as enzyme extract. The assay was carried out with the addition of 2 ml of glutamine, 0.5 ml sodium arsenate, 0.3 ml MnCl_2 , 0.5 ml hydroxylamine, 0.5 ml ADP and 0.2 ml enzyme extract in test tubes. 2 ml of 20 mM Tris-HCl buffer was added instead of glutamine in the blank test tubes. The reaction mixture was incubated for 30 min at 37°C. The reaction was stopped by the addition of 1 ml ferric chloride reagent and the brown colour developed was measured at 540 nm. The standards ranging 100-500 μg γ -glutamyl hydroxamate was prepared by adding 4 ml buffer solution and 1 ml of ferric chloride reagent. GS activity was expressed as $\mu\text{mol } \gamma\text{-glutamyl hydroxamate formed per min per gram of fresh weight}$.

3.8.6.5. Glutamate Synthase (GOGAT)

Glutamate synthase activity in leaves and roots were assayed by a modified method of Matoh *et al.* (1980) as described by Suzuki and Gadal (1982). One ml of the reaction mixture was prepared containing 22.5 μmol phosphate buffer (KH_2PO_4 - Na_2HPO_4), pH 7.3, 5 μmol glutamine, 5 μmol α -ketoglutarate, 0.33 μmol methyl viologen and 9 μmol sodium dithionite dissolved in 0.05 ml of 190 mM NaHCO_3 . The reaction was started by the addition of $\text{Na}_2\text{S}_2\text{O}_4$. After incubation at 30°C for 5 to 15 min, the reaction was stopped by heating the assay tubes at 100°C for 1 min in a water bath. The assay mixture was adjusted at pH 6.95 and centrifuged for 10 min at 2,000g to remove the precipitate. The supernatant was passed through a resin AG 1 x 8 column (3 x 40 mm). After washing with 3 ml of distilled water, glutamate was eluted from the column with 2 ml of 1 N acetic acid. An aliquot of the eluent was incubated with a modified ninhydrin solution and the glutamate was determined by reading the absorbance at 570 nm. One unit of GOGAT enzyme activity was expressed as 1 μmol of glutamate formed per min at 30°C.

3.8.6.6. Glutamate Dehydrogenase (GDH)

One gram of leaves and roots were taken with 5 ml of 100 mM phosphate buffer pH 7.5 containing 1 mM disodium EDTA, 1mM dithioerythritol and 1 per cent of Polyvinyl Pyrrolidone (PVP) and centrifuged at 10,000 g for 30 min at 4°C. The supernatant was collected and used as enzyme extract. The assay was carried out with the addition of 1 ml of potassium phosphate buffer, 0.3 ml of 2-Oxoglutarate, 0.5 ml NH_4Cl , 0.12 ml NADH, 0.2 enzyme extract and 8 ml water. For the blank, 0.3 ml of water was added instead of 2- Oxoglutarate. The mixture was incubated at 37°C for 30 min. The change in the absorbance at 340 nm was recorded and the GDH activity was expressed as μmol NADH oxidized $\text{min}^{-1}\text{g}^{-1}$ fresh weight (Sadasivam and Manickam, 1991).

3.8.6.7. Catalase

Catalase activity in leaves was determined by following the method of Luck (1974). The enzyme extract was prepared by blending the fresh leaves (500 mg) with 0.067 M phosphate buffer (pH 7.0). A known quantity of enzyme extract (0.01-0.04 ml)

was added to the cuvette containing 3 ml H_2O_2 – PO_4 buffer. The change in the absorbance at 240 nm was recorded and expressed as $\mu\text{g H}_2\text{O}_2 \text{ g}^{-1} \text{ min}^{-1}$.

3.8.6.8. Superoxide Dismutase

Superoxide Dismutase activity in leaves was determined by adopting the method of Beauchamp and Fridovich (1971). One gram of fresh leaf tissue was taken in a pre-chilled pestle and mortar containing 10 ml ice-cold 50 mM potassium phosphate buffer (pH 7.8) and homogenated at 10,000 rpm for 10 min at 4°C. 3 ml of reaction mixture containing 50 mM potassium phosphate buffer, pH 7.8, 13 mM methionine, 2 μM riboflavin, 0.1 mM EDTA, 75 μM NBT(Nitroblue tetrazolium) salt and 50 μL of crude enzyme extract. The absorbance at 560 nm was measured and the SOD activity was expressed as enzyme units $\text{mg}^{-1} \text{ protein min}^{-1}$.

3.8.6.9. Peroxidase

Fresh leaves (500 mg) were macerated with 0.1 M phosphate buffer, pH 7.0 in a pre-chilled pestle and mortar at 4°C. The homogenate was centrifuged at 18,000 g at 5°C for 15 min. A known volume of the enzyme extract (0.1 ml) was added to the cuvette containing 3 ml of buffer solution, 0.05 ml guaiacol solution and 0.03 ml hydrogen peroxide solution. Change in the absorbance @ 436 nm was recorded (Malik and Singh, 1980) and expressed as $\Delta 436 \text{ nm g}^{-1} \text{ min}^{-1}$.

3.8.7. Plant analysis

The plant samples were cleaned with water and separated into grains, roots and straw. Plant samples were shade dried and later oven dried at 70°C. After recording the dry weight, each sample was ground in a willey mill and sub-samples were obtained for laboratory analysis. The carbon and nitrogen contents of straw, root and grains were estimated using the standard procedures outlined in the previous section 3.1.8. The biomass carbon content and nitrogen uptake was calculated by multiplying the respective content values with the dry matter production.

3.9. Statistical analysis

The data on various parameters studied during the course of investigation were statistically analyzed as suggested by Gomez and Gomez (1984). The statistical analysis

was performed using the AgRes statistical software (Pascal Intel Software Solutions). Wherever the treatment differences were found significant, critical difference (CD) were worked out at the 5 per cent level of significance with mean separation by least significant difference and denoted by symbol * (** for 1%). Treatment differences that were not significant were denoted as 'NS'.

Experimental Results

CHAPTER 4

RESULTS

Experiments have been conducted systematically to assess the carbon sequestration pattern and methane mitigation potentials in two distinct systems of rice cultivation besides studying the response of rice under elevated levels of CO₂. The results obtained from the two field experiments, laboratory and pot culture studies under elevated levels of CO₂ were statistically analyzed and presented in this chapter.

4.1. Field experiment

4.1.1. Initial soil characteristics

The initial soil characteristics of experimental sites ('D5' and 'B1' Blocks) conducted during summer and *kharif* seasons, respectively at the Wetlands of Tamil Nadu Agricultural University, Coimbatore, is presented in Table 4.1. The soil textural class of both the sites was sandy clay and belongs to Noyyal series. The experimental soils are classified taxonomically as *Typic Ustochrept* according to USDA classification. The initial characterization results revealed that the soils in 'D5' and 'B1' Blocks were slightly alkaline (pH = 8.29 and 8.17) with low soluble salts (EC = 0.38 and 0.31 dS m⁻¹), respectively. Both the soils were high in organic carbon content, low in available nitrogen, medium in available phosphorus and potassium. The enzyme activities viz., phosphatase, dehydrogenase and urease were 11.2 and 9.7 (µg p-nitrophenol g⁻¹ of soil hr⁻¹), 10.5 and 7.3 (µg TPF g⁻¹ of soil hr⁻¹), 8.7 and 10.9 (µg NH₄-N g⁻¹ of soil hr⁻¹) in summer and *kharif* field soils, respectively.

4.1.2. Influence on physico-chemical properties

4.1.2.1. Soil pH

The systems of rice cultivation did not significantly influence the soil pH in both seasons (Table 4.2). The soil pH ranged from 8.07 to 8.26, 8.10 and 8.28 in conventional and SRI systems, respectively during summer season but the values were non-significant. A similar trend of response was observed during *kharif* season. Incremental levels of nitrogen slightly decreased the soil pH, however the values were non-significant. The soil

Table 4.1. Initial soil characteristics of field experiments

Parameters	Summer (2011)	Kharif (2011)
Soil taxonomy	<i>Vertic Ustochrept</i>	<i>Vertic Ustochrept</i>
Soil series	Noyyal	Noyyal
a) Mechanical properties		
Textural class	Sandy clay	Sandy clay
Sand (%)	43.6	43.6
Silt (%)	9.1	9.1
Clay (%)	45.2	45.2
Bulk density (Mg m^{-3})	1.41	1.43
Particle density (Mg m^{-3})	2.60	2.67
b) Physico-chemical properties		
pH	8.29	8.17
EC (dSm^{-1})	0.38	0.31
c) Chemical properties		
Organic carbon (g kg^{-1})	7.51	9.48
Available Nitrogen (kg ha^{-1})	173	202
Available Phosphorus (kg ha^{-1})	15.2	12.6
Available Potassium (kg ha^{-1})	253	246
d) Biological properties		
Bacteria ($\times 10^6$ CFU g^{-1} of soil)	14.0	12.0
Fungi ($\times 10^4$ CFU g^{-1} of soil)	7.0	9.0
Actinomycetes ($\times 10^3$ CFU g^{-1} of soil)	5.0	4.0
Phosphatase activity ($\mu\text{g p-nitrophenol g}^{-1}$ of soil hr^{-1})	11.2	9.7
Dehydrogenase activity ($\mu\text{g TPF g}^{-1}$ of soil hr^{-1})	10.5	7.3
Urease activity ($\mu\text{g NH}_4\text{-N g}^{-1}$ of soil hr^{-1})	8.7	10.9
Microbial biomass carbon (mg kg^{-1})	118	89
Microbial biomass nitrogen (mg kg^{-1})	11.1	12.0

Table 4.2. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil pH

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	8.26	8.21	8.15	8.17	8.13	8.10	8.05	8.06
	N ₅₀	8.25	8.22	8.14	8.17	8.13	8.11	8.04	8.06
	N ₁₀₀	8.23	8.19	8.11	8.15	8.10	8.08	8.01	8.04
	N ₁₅₀	8.21	8.16	8.07	8.14	8.08	8.05	7.97	8.03
S ₂	N ₀	8.28	8.24	8.16	8.18	8.15	8.13	8.06	8.07
	N ₅₀	8.27	8.22	8.15	8.17	8.14	8.11	8.05	8.06
	N ₁₀₀	8.25	8.20	8.12	8.16	8.14	8.09	8.02	8.05
	N ₁₅₀	8.24	8.19	8.10	8.13	8.11	8.09	8.00	8.02
Mean	S ₁	8.24	8.20	8.12	8.16	8.11	8.09	8.02	8.05
	S ₂	8.26	8.21	8.13	8.16	8.14	8.11	8.03	8.05
	N ₀	8.27	8.23	8.16	8.18	8.14	8.12	8.06	8.07
	N ₅₀	8.26	8.22	8.15	8.17	8.14	8.11	8.05	8.06
	N ₁₀₀	8.24	8.20	8.12	8.16	8.12	8.09	8.02	8.05
	N ₁₅₀	8.23	8.18	8.09	8.14	8.10	8.07	7.99	8.03
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.146	NS	0.145	NS	0.144	NS	0.143	NS
	N	0.244	NS	0.243	NS	0.241	NS	0.240	NS
	S at N	0.333	NS	0.332	NS	0.328	NS	0.327	NS
	N at S	0.346	NS	0.344	NS	0.341	NS	0.339	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

pH gradually decreased with the advancement of crop growth stages in both the systems of rice cultivation. The interaction effect between the systems of cultivation and nitrogen levels was non-significant.

4.1.2.2. Soil EC

The soil EC remained constant regardless of systems of cultivation or nitrogen levels or stages of observation (Table 4.3). During summer and *kharif* seasons, the soil EC ranged between 0.39 to 0.45 dS m⁻¹ and 0.28 to 0.35 dS m⁻¹, respectively.

4.1.2.3. Soil redox potential (Eh)

The soil redox potential (Eh) values of SRI were significantly higher than conventional system at tillering and panicle initiation stages in both the seasons (Table 4.4). Conventional system recorded lower mean redox potential (-246, -266, -269 and -128 mV) while the higher values (-197, -246, -269 and -129 mV) were recorded in SRI at tillering, panicle initiation, flowering and maturity stages, respectively. Nitrogen addition did not significantly influence the soil redox potential (Eh) regardless of stages of observation. Among the stages, the reduction was the highest at flowering and the lowest at maturity stages. The interaction effect was non-significant.

4.1.3. Influence on chemical properties

4.1.3.1. Soil organic carbon (SOC)

The soil organic carbon (SOC) contents were significantly higher in conventional system of rice cultivation throughout the cropping period in both the seasons (Table 4.5). At the harvest, the SOC contents were higher in conventional (7.40, 9.55 g kg⁻¹) than SRI systems (7.01, 9.18 g kg⁻¹) during summer and *kharif* seasons, respectively.

Nitrogen fertilization decreased the SOC regardless of system of rice cultivation or stages of observations, but the level of significance was not pronounced. Similar trend of response was observed in both summer and *kharif* seasons. The interaction effect between systems of rice cultivation and nitrogen levels were found non-significant.

4.1.3.2. Dissolved organic carbon (DOC)

The conventional system of rice cultivation recorded significantly higher dissolved organic carbon (DOC) content at all critical stages of crop growth in both the seasons (Table 4.6). During the summer season, DOC content ranged from 341 to 435 mg L⁻¹ and

Table 4.3. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil EC (dSm⁻¹)

Treatments	Summer (2011)					Kharif (2011)				
	Tillering	Panicle Initiation	Flowering	Harvest		Tillering	Panicle Initiation	Flowering	Harvest	
S₁	N ₀	0.39	0.43	0.41		0.30	0.33	0.30	0.29	
	N ₅₀	0.40	0.44	0.43		0.30	0.33	0.30	0.30	
	N ₁₀₀	0.41	0.43	0.44		0.32	0.34	0.33	0.32	
	N ₁₅₀	0.42	0.44	0.43		0.33	0.34	0.33	0.33	
	N ₀	0.40	0.42	0.41		0.31	0.32	0.30	0.28	
S₂	N ₅₀	0.41	0.44	0.42		0.32	0.34	0.31	0.31	
	N ₁₀₀	0.42	0.44	0.43		0.33	0.34	0.32	0.31	
	N ₁₅₀	0.42	0.45	0.42		0.33	0.35	0.31	0.32	
	S ₁	0.41	0.44	0.43		0.31	0.34	0.32	0.31	
	S ₂	0.41	0.44	0.42		0.32	0.34	0.31	0.31	
Mean	N ₀	0.40	0.43	0.41		0.31	0.33	0.30	0.29	
	N ₅₀	0.41	0.44	0.43		0.31	0.34	0.31	0.31	
	N ₁₀₀	0.42	0.44	0.44		0.33	0.34	0.33	0.32	
	N ₁₅₀	0.42	0.45	0.43		0.33	0.35	0.32	0.33	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD	
	S	0.007	NS	0.007	NS	0.005	NS	0.005	NS	NS
	N	0.012	NS	0.012	NS	0.009	NS	0.009	NS	NS
	S at N	0.016	NS	0.017	NS	0.012	NS	0.012	NS	NS
	N at S	0.017	NS	0.018	NS	0.013	NS	0.013	NS	NS
		SEd	CD	SEd	CD	SEd	CD	SEd	CD	

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.4. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil redox potential (mV)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N₀	-244	-264	-268	-127	-242	-267	-265	-130
	N₅₀	-244	-266	-269	-127	-249	-269	-268	-129
	N₁₀₀	-247	-266	-269	-129	-250	-268	-270	-132
	N₁₅₀	-248	-268	-269	-129	-250	-270	-269	-132
S₂	N₀	-196	-243	-268	-129	-200	-245	-267	-132
	N₅₀	-196	-247	-269	-129	-200	-249	-265	-130
	N₁₀₀	-198	-248	-269	-129	-206	-249	-265	-131
	N₁₅₀	-198	-248	-268	-130	-208	-249	-266	-130
Mean	S₁	-246	-266	-269	-128	-248	-269	-268	-131
	S₂	-197	-246	-269	-129	-204	-248	-266	-131
	N₀	-220	-253	-268	-128	-221	-256	-266	-131
	N₅₀	-220	-256	-269	-128	-225	-259	-267	-130
	N₁₀₀	-223	-257	-269	-129	-228	-259	-268	-132
	N₁₅₀	-223	-258	-269	-130	-229	-260	-268	-131
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	3.6	11.7**	4.4	14.0*	3.7	11.8**	4.4	14.1*
	N	6.7	NS	7.6	NS	6.7	NS	7.6	NS
	S at N	9.1	NS	10.3	NS	9.1	NS	10.3	NS
	N at S	9.6	NS	10.8	NS	9.6	NS	10.8	NS

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.5. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil organic carbon (g kg⁻¹)

Treatments		Summer (2011)				Kharif (2011)								
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest					
S ₁	N ₀	7.91	7.79	7.73	7.50	9.98	9.91	9.87	9.69					
	N ₅₀	7.89	7.75	7.69	7.41	9.92	9.85	9.75	9.58					
	N ₁₀₀	7.85	7.71	7.62	7.38	9.87	9.79	9.70	9.50					
	N ₁₅₀	7.83	7.68	7.55	7.29	9.79	9.71	9.61	9.44					
S ₂	N ₀	7.34	7.29	7.25	7.11	9.41	9.33	9.30	9.28					
	N ₅₀	7.30	7.25	7.19	7.04	9.36	9.28	9.25	9.21					
	N ₁₀₀	7.28	7.22	7.15	6.99	9.30	9.20	9.18	9.15					
	N ₁₅₀	7.24	7.18	7.13	6.91	9.26	9.14	9.11	9.08					
Mean	S ₁	7.87	7.73	7.65	7.40	9.89	9.82	9.73	9.55					
	S ₂	7.29	7.23	7.18	7.01	9.33	9.24	9.21	9.18					
	N ₀	7.63	7.54	7.49	7.31	9.70	9.62	9.59	9.48					
	N ₅₀	7.59	7.50	7.44	7.23	9.64	9.57	9.50	9.39					
	N ₁₀₀	7.56	7.46	7.39	7.19	9.59	9.50	9.44	9.32					
	N ₁₅₀	7.53	7.43	7.34	7.10	9.53	9.43	9.36	9.26					
		SEd	CD	SEd	CD	SEd	CD	SEd	CD					
S	0.130	0.416*	0.129	0.412*	0.128	0.407*	0.124	0.397**	0.165	0.528*	0.163	0.521*	0.096	0.305*
N	0.227	NS	0.224	NS	0.221	NS	0.215	NS	0.286	NS	0.282	NS	0.252	NS
S at N	0.307	NS	0.303	NS	0.300	NS	0.291	NS	0.388	NS	0.383	NS	0.323	NS
N at S	0.320	NS	0.316	NS	0.313	NS	0.304	NS	0.405	NS	0.399	NS	0.356	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.6. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on dissolved organic carbon (mg L⁻¹)

Treatments		Summer (2011)				Kharif (2011)									
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest						
S ₁	N ₀	341	370	390	369	370	388	407	350						
	N ₅₀	360	378	405	384	389	396	422	353						
	N ₁₀₀	370	390	421	400	399	408	438	357						
	N ₁₅₀	382	405	435	414	411	423	452	359						
	N ₀	322	342	364	343	351	360	381	345						
S ₂	N ₅₀	335	355	379	358	364	373	396	349						
	N ₁₀₀	344	367	391	370	373	385	408	352						
	N ₁₅₀	352	379	401	380	381	397	418	355						
Mean	S ₁	363	386	413	392	392	404	430	355						
	S ₂	338	361	384	363	367	379	401	350						
	N ₀	332	356	377	356	361	374	394	348						
	N ₅₀	348	367	392	371	377	385	409	351						
	N ₁₀₀	357	379	406	385	386	397	423	355						
	N ₁₅₀	367	392	418	397	396	410	435	357						
		SEd	CD	SEd	CD	SEd	CD	SEd	CD						
	S	6.3	19.9*	6.6	21.1*	6.7	21.4*	6.7	21.6*	6.9	22.1*	7.4	23.5*	6.3	NS
	N	10.5	21.9*	11.1	23.4*	11.3	23.7**	11.3	23.8*	11.7	24.5*	12.4	26.0*	10.5	NS
	S at N	14.3	NS	15.2	NS	16.2	NS	15.3	NS	15.4	NS	16.9	NS	14.3	NS
	N at S	14.8	NS	15.8	NS	16.8	NS	15.9	NS	16.0	NS	17.5	NS	14.8	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

322 to 401 mg L⁻¹ in conventional and SRI systems, respectively. It was observed that in *kharif* season, the DOC content ranged from 350 to 452 mg L⁻¹ and 345 to 418 mg L⁻¹ in conventional and SRI treatments, respectively.

At all the critical stages of crop growth, the highest DOC contents were observed in N₁₅₀ and the lowest in control (N₀). Among the different stages of observation, the highest DOC content was recorded at the flowering stage irrespective of the seasons. The interaction effect was found non-significant.

4.1.3.3. Soil microbial biomass carbon

The SRI cultivation recorded significantly higher soil microbial biomass carbon during summer and *kharif* seasons (Table 4.7). At all the stages of observation, the microbial biomass carbon were consistently higher in SRI (175 to 274, 137 to 229 mg kg⁻¹ soil) than conventional system (139 to 246, 101 to 201 mg kg⁻¹ soil) in both summer and *kharif* seasons, respectively. In SRI, the mean microbial biomass carbon increased by 16 to 13 per cent and 20 to 17 per cent over the conventional system during the growth stages in both summer and *kharif* seasons, respectively.

Nitrogen fertilization significantly increased the soil microbial biomass in both SRI and conventional systems. At all the stages of observation, the highest microbial biomass carbon was observed in N₁₅₀ that ranged from 212 to 274, 174 to 229 mg kg⁻¹ soil and the lowest values in control (N₀) that ranged from 139 to 217, 101 to 172 mg kg⁻¹ soil in summer and *kharif* seasons, respectively. Among the critical stages, the flowering registered the highest microbial biomass in both the seasons. The interaction effect was non-significant.

4.1.3.4. Soil microbial biomass nitrogen

Soil microbial biomass nitrogen was significantly higher in SRI than conventional system in both seasons (Table 4.8). The microbial biomass nitrogen in SRI ranged from 16.4 to 32.8 mg kg⁻¹, 13.0 to 27.4 mg kg⁻¹ soil and in conventional system it ranged from 12.5 to 30.1 mg kg⁻¹ soil, 9.4 to 24.6 mg kg⁻¹ soil in summer and *kharif* seasons, respectively.

Table 4.7. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil microbial biomass carbon (mg kg⁻¹ soil)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N₀	139	176	192	151	101	133	147	114
	N₅₀	165	189	204	171	127	146	159	134
	N₁₀₀	182	222	231	190	144	179	186	153
	N₁₅₀	212	237	246	214	174	194	201	177
S₂	N₀	175	202	217	179	137	159	172	142
	N₅₀	194	225	238	196	156	182	193	159
	N₁₀₀	215	243	255	219	177	200	210	182
	N₁₅₀	227	259	274	231	189	216	229	194
Mean	S₁	175	206	218	182	137	163	173	145
	S₂	203	232	246	206	165	189	201	169
	N₀	157	189	205	165	119	146	160	128
	N₅₀	180	207	221	184	142	164	176	147
	N₁₀₀	199	233	243	205	161	190	198	168
	N₁₅₀	220	248	260	223	182	205	215	186
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	3.9	12.3**	4.3	13.8**	3.2	10.2**	3.7	11.9**
	N	5.5	11.6**	6.4	13.5**	4.4	9.3**	5.5	11.6**
	S at N	7.8	NS	9.0	NS	6.3	NS	7.7	NS
	N at S	7.8	NS	9.1	NS	6.3	NS	7.8	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.8. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation and different levels of nitrogen on soil microbial biomass nitrogen (mg kg⁻¹soil)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N₀	12.5	16.3	20.5	14.8	9.4	12.3	15.7	11.2
	N₅₀	14.7	22.4	27.7	19.5	11.3	17.3	21.6	15.3
	N₁₀₀	21.2	23.4	29.1	23.3	16.8	18.9	23.4	18.8
	N₁₅₀	23.1	27.2	30.1	24.3	19.0	22.3	24.6	20.1
S₂	N₀	16.9	19.4	21.6	16.4	13.2	15.3	17.1	13.0
	N₅₀	22.0	25.8	27.7	22.6	17.7	20.9	22.5	18.3
	N₁₀₀	25.3	28.7	30.7	25.5	20.8	23.6	25.3	21.2
	N₁₅₀	26.9	32.0	32.8	27.3	22.4	26.7	27.4	22.9
Mean	S₁	17.9	22.3	26.8	20.5	14.1	17.7	21.3	16.4
	S₂	22.8	26.5	28.2	22.9	18.5	21.6	23.1	18.9
	N₀	14.7	17.9	21.0	15.6	11.3	13.8	16.4	12.1
	N₅₀	18.3	24.1	27.7	21.0	14.5	19.1	22.1	16.8
	N₁₀₀	23.2	26.1	29.9	24.4	18.8	21.3	24.4	20.0
	N₁₅₀	25.0	29.6	31.4	25.8	20.7	24.5	26.0	21.5
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.44	1.40**	0.53	1.60**	0.36	1.15**	0.44	1.39*
	N	0.60	1.25**	0.81	1.51**	0.48	1.01**	0.65	1.37**
	S at N	0.85	2.04*	1.13	NS	0.69	1.66*	0.91	NS
	N at S	0.84	1.78*	1.15	NS	0.68	1.43*	0.93	NS

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Nitrogen fertilization significantly increased the soil microbial biomass nitrogen in both seasons. The highest microbial biomass was registered in N₁₅₀ (23.1 to 32.8 mg kg⁻¹ soil, 19.0 to 27.4 mg kg⁻¹ soil) and lowest in N₀ (12.5 to 21.6 mg kg⁻¹ soil, 9.4 to 17.1 mg kg⁻¹ soil) during summer and *kharif* seasons, respectively. Among the stages, the flowering had the highest microbial biomass and lowest at active tillering. The interaction effect was significant only at the tillering stage.

4.1.3.5. Humic acid fractions

The humic acid fraction was measured significantly higher in SRI (14.4 to 20.9 per cent of OM, 12.0 to 18.1 per cent of OM) than conventional (11.9 to 19.8 per cent of OM, 9.7 to 17.1 per cent of OM) in summer and *kharif* seasons, respectively (Table 4.9).

Nitrogen fertilized soils significantly increased the humic acid fraction in both the seasons. N₁₅₀ recorded the highest humic acid fraction (16.4 to 20.9 per cent of OM, 13.9 to 18.1 per cent of OM) and the lowest in control (11.9 to 17.6 per cent of OM, 9.7 to 14.9 per cent of OM) during summer and *kharif* seasons, respectively. Humic acid fraction was the highest at harvest and the lowest at active tillering. The interaction effect was absent.

4.1.3.6. Fulvic acid fractions

The SRI had significantly higher fulvic acid fraction than conventional system. In summer season, fulvic acid fraction ranged from 19.4 to 27.6 per cent of OM and 16.0 to 26.5 per cent of OM in SRI and conventional systems, respectively (Table 4.10). Similar trend was observed in *kharif*.

Nitrogen levels significantly increased the fulvic acid fraction. During the critical stages of crop growth, the fulvic acid fractions were consistently higher in N₁₅₀ (21.4 to 27.6, 19.1 to 25.6 per cent of OM) and the lowest in N₀ (16.0 to 22.3, 13.4 to 20.0 per cent of OM) during summer and *kharif* seasons, respectively. Among the stages, the highest fulvic acid fraction was recorded at the harvest regardless of the seasons. The interaction effect was non-significant.

4.1.3.7. Humin fractions

Conventional system had slightly higher soil humin fraction than SRI but the values were non-significant (Table 4.11). At the harvest, the mean humin fraction

Table 4.9. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil humic acid fractions (% of OM)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	11.9	12.6	13.3	14.3	9.7	10.3	10.9	11.8
	N ₅₀	13.5	14.3	14.9	16.1	11.1	11.9	12.5	13.6
	N ₁₀₀	14.8	15.9	16.3	17.9	12.4	13.4	13.8	15.2
	N ₁₅₀	16.4	17.7	18.5	19.8	13.9	15.1	15.8	17.1
S ₂	N ₀	14.4	15.3	15.9	17.6	12.0	12.8	13.4	14.9
	N ₅₀	15.0	15.9	16.6	18.4	12.6	13.5	14.1	15.7
	N ₁₀₀	16.4	17.7	18.4	19.2	13.9	15.1	15.8	16.5
	N ₁₅₀	17.0	18.3	19.0	20.9	14.5	15.7	16.3	18.1
Mean	S ₁	14.1	15.1	15.7	17.0	11.8	12.7	13.3	14.4
	S ₂	15.7	16.8	17.5	19.0	13.3	14.3	14.9	16.3
	N ₀	13.1	14.0	14.6	15.9	10.8	11.6	12.2	13.4
	N ₅₀	14.3	15.1	15.7	17.3	11.9	12.7	13.3	14.7
	N ₁₀₀	15.6	16.8	17.4	18.6	13.2	14.2	14.8	15.9
	N ₁₅₀	16.7	18.0	18.7	20.4	14.2	15.4	16.1	17.6
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.29	0.94**	0.32	1.01**	0.25	0.80**	0.27	0.87**
	N	0.44	0.92**	0.47	0.99**	0.37	0.77**	0.39	0.83**
	S at N	0.61	NS	0.65	NS	0.51	NS	0.56	NS
	N at S	0.62	NS	0.66	NS	0.52	NS	0.59	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.10. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil fulvic acid fractions (% of OM)

Treatments		Summer (2011)				Kharif (2011)										
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest							
S ₁	N ₀	16.0	17.1	17.8	19.0	13.4	14.5	15.2	16.5							
	N ₅₀	18.2	19.9	20.9	21.9	15.7	17.5	18.6	19.6							
	N ₁₀₀	19.7	21.7	23.2	24.9	17.3	19.4	21.0	22.7							
	N ₁₅₀	21.4	23.6	25.5	26.5	19.1	21.4	23.5	24.4							
S ₂	N ₀	19.4	20.6	21.5	22.3	17.0	18.2	19.2	20.0							
	N ₅₀	20.6	22.3	23.4	24.7	18.3	20.0	21.2	22.5							
	N ₁₀₀	22.1	23.8	25.2	26.2	19.9	21.7	23.1	24.1							
	N ₁₅₀	23.3	25.9	26.3	27.6	21.2	23.8	24.3	25.6							
Mean	S ₁	18.8	20.6	21.9	23.1	16.4	18.2	19.6	20.8							
	S ₂	21.4	23.2	24.1	25.2	19.1	20.9	22.0	23.1							
	N ₀	17.7	18.9	19.6	20.7	15.2	16.4	17.2	18.2							
	N ₅₀	19.4	21.1	22.1	23.3	17.0	18.8	19.9	21.0							
	N ₁₀₀	20.9	22.8	24.2	25.6	18.6	20.6	22.1	23.4							
	N ₁₅₀	22.3	24.7	25.9	27.1	20.1	22.6	23.9	25.0							
		SEd	CD	SEd	CD	SEd	CD	SEd	CD							
S	0.39	1.27**	0.43	1.38**	0.46	1.46*	0.48	1.52*	0.36	1.14**	0.42	1.34**	0.44	1.40**		
N	0.59	1.24**	0.64	1.35**	0.67	1.41**	0.71	1.49**	0.52	1.09**	0.57	1.21**	0.61	1.28**	0.64	1.35**
S at N	0.82	NS	0.89	NS	0.95	NS	0.99	NS	0.73	NS	0.81	NS	0.56	NS	0.90	NS
N at S	0.83	NS	0.91	NS	0.95	NS	1.00	NS	0.74	NS	0.81	NS	0.56	NS	0.91	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.11. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation and different levels of nitrogen on soil humin fractions (% of OM)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	44.73	44.88	44.54	43.21	47.22	47.60	47.32	46.96
	N ₅₀	44.71	44.02	44.55	43.11	47.47	46.97	47.64	47.02
	N ₁₀₀	44.16	44.46	44.08	42.90	47.09	47.71	47.39	46.97
	N ₁₅₀	44.03	44.03	43.97	43.30	47.21	47.55	47.63	47.58
	N ₀	44.04	43.98	43.98	43.34	46.89	47.08	47.18	47.41
S ₂	N ₅₀	44.13	44.12	44.13	42.69	47.11	47.24	47.38	47.26
	N ₁₀₀	44.12	43.64	43.46	42.04	47.34	47.13	47.06	47.63
	N ₁₅₀	44.10	43.43	42.87	42.06	47.44	47.05	47.52	46.89
Mean	S ₁	44.41	44.35	44.29	43.13	47.25	47.46	47.49	47.13
	S ₂	44.10	43.79	43.61	42.53	47.19	47.12	47.28	47.30
	N ₀	44.39	44.43	44.26	43.27	47.06	47.34	47.25	47.18
	N ₅₀	44.42	44.07	44.34	42.90	47.29	47.11	47.51	47.14
	N ₁₀₀	44.14	44.05	43.77	42.47	47.21	47.42	47.22	47.30
	N ₁₅₀	44.07	43.73	43.42	42.68	47.32	47.30	47.57	47.23
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.78	NS	0.78	NS	0.83	NS	0.84	NS
	N	1.31	NS	1.31	NS	1.40	NS	1.41	NS
	S at N	1.79	NS	1.78	NS	1.91	NS	1.92	NS
	N at S	1.86	NS	1.85	NS	1.98	NS	1.99	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

contents were 43.13, 47.13 and 42.53, 47.30 in conventional and SRI during summer and *kharif* seasons, respectively. Four stages of sampling revealed that the nitrogen fertilization also did not influence the humin fractions in both the seasons.

4.1.3.8. Non-Humic fractions

Non-humic fractions were significantly higher in conventional (10.36 to 27.33, 10.90 to 29.71 per cent of OM) than SRI (9.43 to 22.18, 9.41 to 24.11 per cent of OM) during summer and *kharif* seasons, respectively (Table 4.12).

Application of nitrogen significantly decreased the non-humic fractions in both the seasons. The control (N_0) recorded the highest non-humic fractions (16.78 to 27.33, 17.66 to 29.71 per cent of OM) and lowest in N_{150} (9.43 to 18.25, 9.41 to 19.84 per cent of OM) during summer and *kharif* seasons, respectively. A declining trend in the non-humic acid fractions was observed throughout the crop growth stages in both the seasons. The interaction effect was significant only at flowering and harvest stages in both seasons.

4.1.3.9. Glomalin

Higher glomalin contents ranging from 0.66 to 0.73 and 0.59 to 0.62 mg g⁻¹ were recorded in SRI compared to conventional system (0.32 to 0.41, 0.19 to 0.24 mg g⁻¹) in summer and *kharif* seasons, respectively (Table 4.13).

Nitrogen fertilization significantly increased the glomalin contents in both seasons. N_{150} recorded the highest mean glomalin contents (0.57, 0.43 mg g⁻¹) and the lowest (0.49, 0.39 mg g⁻¹) in control (N_0). The interaction effect was non-significant.

4.1.3.10. Soil available nitrogen

The available nitrogen contents of SRI soil were significantly higher than conventional system of rice cultivation regardless of stages of observation (Table 4.14). In summer, the highest soil available nitrogen contents (162 to 226 kg ha⁻¹) were recorded at tillering stage in SRI which decreased with the progression of stages of growth registering the lowest values at harvest (133 to 174 kg ha⁻¹). Similar trend of response was observed for conventional system at tillering (156 to 219 kg ha⁻¹) and harvest (122 to 158 kg ha⁻¹) stages, but the values were lower than SRI.

Table 4.12. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil non-humic fractions (% of OM)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N₀	27.33	25.37	24.41	23.48	29.71	27.58	26.54	24.72
	N₅₀	23.61	21.77	19.62	18.80	25.66	23.67	21.33	19.79
	N₁₀₀	21.38	17.92	16.41	14.32	23.24	19.48	17.84	15.07
	N₁₅₀	18.25	14.71	12.03	10.36	19.84	15.99	13.07	10.90
S₂	N₀	22.18	20.13	18.58	16.78	24.11	21.88	20.20	17.66
	N₅₀	20.21	17.67	15.94	14.27	21.97	19.21	17.32	14.56
	N₁₀₀	17.36	14.86	12.94	12.51	18.87	16.15	14.06	11.73
	N₁₅₀	15.56	12.37	11.80	9.43	16.92	13.45	11.84	9.41
Mean	S₁	22.64	19.94	18.12	16.74	24.61	21.68	19.69	17.62
	S₂	18.83	16.26	14.81	13.25	20.47	17.67	15.85	13.34
	N₀	24.76	22.75	21.50	20.13	26.91	24.73	23.37	21.19
	N₅₀	21.91	19.72	17.78	16.54	23.82	21.44	19.33	17.18
	N₁₀₀	19.37	16.39	14.67	13.41	21.05	17.82	15.95	13.40
	N₁₅₀	16.91	13.54	11.91	9.89	18.38	14.72	12.45	10.16
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.304	0.970**	0.251	0.801**	0.331	1.054**	0.229	0.729**
	N	0.638	1.341**	0.564	1.186**	0.694	1.458**	0.560	1.177**
	S at N	0.839	NS	0.735	NS	0.912	NS	0.724	1.599**
	N at S	0.903	NS	0.798	NS	0.982	NS	0.793	1.665**
	S								
	N								
	S at N								
	N at S								

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.13. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation and different levels of nitrogen on glomalin (mg g⁻¹)

Treatments		Summer (2011)	<i>Kharif</i> (2011)
S₁	N₀	0.32	0.19
	N₅₀	0.35	0.23
	N₁₀₀	0.39	0.24
	N₁₅₀	0.41	0.24
S₂	N₀	0.66	0.59
	N₅₀	0.69	0.60
	N₁₀₀	0.71	0.62
	N₁₅₀	0.73	0.61
Mean	S₁	0.37	0.23
	S₂	0.70	0.61
	N₀	0.49	0.39
	N₅₀	0.52	0.42
	N₁₀₀	0.55	0.43
	N₁₅₀	0.57	0.43

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.14. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil available nitrogen (kg ha⁻¹)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	156	142	130	122	190	182	169	158
	N ₅₀	177	161	148	138	211	200	185	176
	N ₁₀₀	194	173	157	146	228	211	198	186
	N ₁₅₀	219	191	171	158	253	229	210	194
	N ₀	162	149	139	133	196	189	177	168
S ₂	N ₅₀	185	170	159	150	219	208	195	185
	N ₁₀₀	204	186	173	163	238	225	211	200
	N ₁₅₀	226	202	185	174	260	238	222	209
Mean	S ₁	187	167	152	141	221	206	191	179
	S ₂	194	177	164	155	228	215	201	191
	N ₀	159	146	135	128	193	186	173	163
	N ₅₀	181	166	154	144	215	204	190	181
	N ₁₀₀	199	180	165	155	233	218	205	193
	N ₁₅₀	223	197	178	166	257	234	216	202
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	3.7	NS	3.3	NS	4.3	NS	3.6	NS
	N	5.6	11.9**	5.1	10.6**	6.7	14.0**	5.8	12.3
	S at N	7.8	NS	7.0	NS	9.3	NS	8.0	NS
	N at S	8.0	NS	7.1	NS	9.4	NS	8.2	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Incremental levels of nitrogen addition linearly increased the available nitrogen contents of both SRI and conventional systems irrespective of growth stages or seasons. Similar response was observed in both the seasons. The interaction effect was not pronounced.

4.1.3.11. Ammoniacal nitrogen

Ammoniacal nitrogen contents were consistently and significantly higher in conventional system compared to SRI in both the seasons (Table 4.15). Higher ammoniacal nitrogen contents (11.2 to 53.2, 14.5 to 56.3 mg kg⁻¹) were registered in conventional system than SRI (10.8 to 38.7, 14.1 to 41.8 mg kg⁻¹) during summer and *kharif* seasons, respectively.

Nitrogen fertilized soils had the highest ammoniacal nitrogen in both the seasons. N₁₅₀ registered highest ammoniacal nitrogen and the values ranged from 32.4 to 53.2 mg kg⁻¹ and 35.7 to 56.3 mg kg⁻¹ during summer and *kharif* seasons, respectively. The lowest ammoniacal nitrogen contents were observed in control (N₀) and it ranged from 10.8 to 20.9 mg kg⁻¹ and 14.1 to 23.5 mg kg⁻¹ in summer and *kharif* seasons, respectively.

Among the stages of observation, the flowering stage recorded the highest ammoniacal nitrogen contents in all the nitrogen fertilized soils. But in both the seasons, the control (N₀) registered the highest ammoniacal nitrogen at tillering stage. The interaction effect was significant at panicle initiation and flowering stages.

4.1.3.12. Nitrate nitrogen

At all the stages of observation, nitrate nitrogen was significantly higher in SRI compared to conventional system (Table 4.16). Highest nitrate nitrogen contents (11.4 to 26.9, 12.2 to 28.3 mg kg⁻¹) were registered in SRI and lowest (8.1 to 23.7, 8.9 to 25.1 mg kg⁻¹) were observed in conventional system during summer and *kharif* seasons, respectively.

Nitrogen fertilization significantly increased the soil nitrate nitrogen contents in both the seasons. N₁₅₀ registered the highest nitrate nitrogen and the values ranged from 16.3 to 26.9 mg kg⁻¹ and 17.1 to 28.3 mg kg⁻¹ during summer and *kharif* seasons, respectively. The lowest nitrate nitrogen contents were observed in control (N₀) and it ranged from 8.1 to 15.5 mg kg⁻¹ and 8.9 to 16.9 mg kg⁻¹ in summer and *kharif* seasons, respectively.

Among the critical stages, the tillering had the highest soil nitrate nitrogen regardless of the systems of cultivation. The interaction effect was significant at panicle initiation and flowering stages.

Table 4.15. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil ammoniacal nitrogen (mg kg⁻¹)

Treatments		Summer (2011)				Kharif (2011)										
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest							
S ₁	N ₀	20.9	18.1	15.6	11.2	23.5	21.0	18.7	14.5							
	N ₅₀	32.1	35.0	42.8	25.8	34.7	37.9	45.9	29.1							
	N ₁₀₀	35.0	36.6	47.4	30.5	37.6	39.5	50.5	33.8							
	N ₁₅₀	44.3	45.3	53.2	32.6	46.9	48.2	56.3	35.9							
S ₂	N ₀	19.2	16.4	13.9	10.8	21.8	19.3	17.0	14.1							
	N ₅₀	26.1	26.5	28.2	24.3	28.7	29.4	31.3	27.6							
	N ₁₀₀	32.7	34.2	35.4	29.9	35.3	37.1	38.5	33.2							
	N ₁₅₀	38.3	38.5	38.7	32.4	40.9	41.4	41.8	35.7							
Mean	S ₁	33.1	33.8	39.8	25.0	35.7	36.7	42.9	28.3							
	S ₂	29.1	28.9	29.1	24.4	31.7	31.8	32.2	27.7							
	N ₀	20.1	17.3	14.8	11.0	22.7	20.2	17.9	14.3							
	N ₅₀	29.1	30.8	35.5	25.1	31.7	33.7	38.6	28.4							
	N ₁₀₀	33.9	35.4	41.4	30.2	36.5	38.3	44.5	33.5							
	N ₁₅₀	41.3	41.9	46.0	32.5	43.9	44.8	49.1	35.8							
		SEd	CD	SEd	CD	SEd	CD	SEd	CD							
S	0.62	2.00**	0.64	2.04**	0.71	2.28**	0.51	NS	0.67	2.15**	0.69	2.21**	0.77	2.45**	0.57	NS
N	0.94	1.98**	0.95	2.01**	1.07	2.25**	0.75	1.57**	1.02	2.14**	1.04	2.18**	1.16	2.43**	0.84	1.76**
S at N	1.31	NS	1.33	3.14**	1.49	3.51**	1.05	NS	1.42	NS	1.45	3.40**	1.61	3.79**	1.18	NS
N at S	1.33	NS	1.35	2.84**	1.51	3.18**	1.06	NS	1.44	NS	1.47	3.09**	1.64	3.44**	1.19	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.16. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil nitrate nitrogen (mg kg⁻¹)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N₀	11.8	11.1	8.9	8.1	13.2	12.4	10	8.9
	N₅₀	15.6	13.5	12.9	11.6	17	14.8	14	12.4
	N₁₀₀	22.3	15.6	14.6	13.7	23.7	16.9	15.7	14.5
	N₁₅₀	23.7	17.8	16.5	16.3	25.1	19.1	17.6	17.1
S₂	N₀	15.5	12.8	12.3	11.4	16.9	14.1	13.4	12.2
	N₅₀	20.3	17.5	14.9	14.0	21.7	18.8	16	14.8
	N₁₀₀	24.1	20.6	19.2	16.6	25.5	21.9	20.3	17.4
	N₁₅₀	26.9	24.7	21.9	18.5	28.3	26.0	23.0	19.3
Mean	S₁	18.4	14.5	13.2	12.4	19.8	15.8	14.3	13.2
	S₂	21.7	18.9	17.1	15.1	23.1	20.2	18.2	15.9
	N₀	13.7	12.0	10.6	9.8	15.1	13.3	11.7	10.6
	N₅₀	18.0	15.5	13.9	12.8	19.4	16.8	15.0	13.6
	N₁₀₀	23.2	18.1	16.9	15.2	24.6	19.4	18.0	16.0
	N₁₅₀	25.3	21.3	19.2	17.4	26.7	22.6	20.3	18.2
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.42	1.36**	0.32	0.29	0.45	0.37	0.34	0.31
	N	0.59	1.24**	0.45	0.40	0.63	0.53	0.48	0.43
	S at N	0.84	NS	0.64	0.58	0.89	0.75	0.68	0.61
	N at S	0.84	NS	0.63	0.57	0.89	0.75	0.68	0.60

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

4.1.4. Influence on biological properties

4.1.4.1. Bacterial population

Bacterial population was significantly higher in SRI than conventional system at all stages of observation (Table 4.17). Soil bacterial population in SRI ranged from 14.8 to 37.4 and 10.8 to 29.5 $\times 10^6$ CFU g^{-1} of dry soil) and in conventional system it ranged from 12.0 to 33.1 and 8.8 to 26.1 $\times 10^6$ CFU g^{-1} of dry soil) during summer and *kharif* seasons, respectively.

Application of nitrogen significantly increased the soil bacterial population. N_{150} registered the highest bacterial population ranging from 22.9 to 37.4 and 16.0 to 29.5 $\times 10^6$ CFU g^{-1} of dry soil) during summer and *kharif* seasons, respectively. The lowest bacterial population ranging from 12.0 to 20.1 and 8.8 to 15.9 $\times 10^6$ CFU g^{-1} of dry soil was observed in control (N_0) during summer and *kharif* seasons, respectively.

The highest bacterial population was observed at panicle initiation stage and decreased thereafter in both the seasons. The interaction effect of systems of rice cultivation and nitrogen levels was significant at active tillering.

4.1.4.2. Fungal population

SRI had significantly higher soil fungal population than conventional system irrespective of stages of observation or seasons (Table 4.18). During summer season, the fungal population varied from 10.0 $\times 10^6$ CFU g^{-1} of dry soil at the harvest stage to 25.6 $\times 10^6$ CFU g^{-1} of dry soil at panicle initiation in SRI soils. Fungal population in conventional system ranged from 9.9 $\times 10^6$ CFU g^{-1} of dry soil at the harvest to 23.0 $\times 10^6$ CFU g^{-1} of dry soil at panicle initiation stage. Similar was the case observed during *kharif* season.

Incremental levels of nitrogen addition registered increased the fungal population at all stages of crop growth. The highest fungal population was observed in N_{150} and the mean values ranged between 17.6 $\times 10^6$ CFU g^{-1} of dry soil and 24.3 $\times 10^6$ CFU g^{-1} of dry soil in summer and between 18.5 $\times 10^6$ CFU g^{-1} of dry soil and 23.6 $\times 10^6$ CFU g^{-1} of dry soil during *kharif* season. At all stages, N_0 recorded the lowest fungal population ranging from 10.0 to 13.0 $\times 10^6$ CFU g^{-1} of dry soil and 11.4 to 12.7 $\times 10^6$ CFU g^{-1} of dry soil during summer and *kharif* seasons, respectively. Among the critical stages, panicle initiation recorded highest fungal population and the lowest at tillering stage in both seasons.

Table 4.17. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil bacterial population ($\times 10^6$ CFU g⁻¹ of dry soil)

Treatments	Summer (2011)				Kharif (2011)			
	Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N ₀	12.2	16.7	15.0	12.0	13.2	10.7	8.8
	N ₅₀	14.8	26.6	23.1	19.0	21.0	16.4	13.9
	N ₁₀₀	19.7	30.5	26.5	22.0	24.1	18.8	16.1
	N ₁₅₀	22.9	33.1	31.6	25.1	26.1	22.4	18.3
S₂	N ₀	15.5	20.1	17.4	14.8	15.9	12.8	10.8
	N ₅₀	21.4	32.7	27.3	23.2	25.8	19.4	16.9
	N ₁₀₀	23.8	34.0	30.8	26.0	26.9	21.9	19.0
	N ₁₅₀	26.3	37.4	34.0	29.4	29.5	24.1	21.5
Mean	S ₁	17.4	26.7	24.1	19.5	21.1	17.1	14.3
	S ₂	21.8	31.1	27.4	23.4	24.5	19.5	17.0
	N ₀	13.9	18.4	16.2	13.4	14.5	11.7	9.8
	N ₅₀	18.1	29.7	25.2	21.1	23.4	17.9	15.4
	N ₁₀₀	21.8	32.3	28.7	24.0	25.5	20.3	17.5
	N ₁₅₀	24.6	35.3	32.8	27.3	27.8	23.3	19.9
		SEd	CD	SEd	CD	SEd	CD	SEd
	S	0.42	1.35**	0.61	1.94**	0.48	1.53**	0.33
	N	0.57	1.21**	0.85	1.78**	0.67	1.41*	0.46
	S at N	0.82	1.97*	1.20	NS	0.95	NS	0.66
	N at S	0.81	1.71*	1.20	NS	0.95	NS	0.65
				1.07	NS	0.76	NS	0.65
				1.75**	1.46**	0.39	1.25**	1.06**
				0.76	1.33**	0.54	1.14**	0.97*
				1.08	NS	0.77	NS	NS
				1.07	NS	0.76	NS	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.18. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil fungal population ($\times 10^4$ CFU g⁻¹ of dry soil)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N₀	10.3	12.3	10.8	9.9	10.4	11.9	11.0	13.3
	N₅₀	12.4	18.2	16.1	13.9	13.0	17.7	16.4	15.8
	N₁₀₀	13.8	19.1	17.4	14.8	14.5	18.5	17.8	15.4
	N₁₅₀	16.5	23.0	20.3	18.5	17.4	22.3	20.7	21.0
S₂	N₀	11.7	13.8	11.8	10.0	12.3	13.4	12.0	11.4
	N₅₀	16.0	20.7	17.9	16.4	16.8	20.1	18.3	18.6
	N₁₀₀	17.4	24.5	20.0	17.8	18.3	23.8	20.4	20.2
	N₁₅₀	18.6	25.6	22.3	18.7	19.6	24.9	22.8	21.3
Mean	S₁	13.2	18.1	16.1	14.3	13.8	17.6	16.5	16.4
	S₂	15.9	21.2	18.0	15.7	16.8	20.6	18.4	17.9
	N₀	11.0	13.0	11.3	10.0	11.4	12.7	11.5	12.4
	N₅₀	14.2	19.5	17.0	15.1	14.9	18.9	17.4	17.2
	N₁₀₀	15.6	21.8	18.7	16.3	16.4	21.2	19.1	17.8
	N₁₅₀	17.6	24.3	21.3	18.6	18.5	23.6	21.8	21.2
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.30	0.97**	0.41	1.31**	0.32	1.02**	0.36	1.15**
	N	0.42	0.90**	0.58	1.22**	0.44	0.94**	0.51	1.08**
	S at N	0.61	1.44*	0.82	1.95*	0.63	1.49**	0.72	1.69**
	N at S	0.61	1.27*	0.82	1.72*	0.64	1.32**	0.73	1.51**

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

The interaction effect was significant at tillering, panicle initiation and harvest stages during summer. In *kharif* season, the interaction effect was significant only at panicle initiation and harvest stages.

4.1.4.3. Actinomycetes population

Actinomycetes population was higher in SRI than conventional system. The mean actinomycetes population varied from 5.7 to 11.1 x 10³ CFU g⁻¹ of dry soil and 4.5 to 7.8 x 10³ CFU g⁻¹ of dry soil in SRI and conventional soils, respectively, during summer (Table 4.19). In *kharif* season, the mean population of actinomycetes population ranged from 4.6 to 8.4 x 10³ CFU g⁻¹ of dry soil and 3.7 to 5.8 x 10³ CFU g⁻¹ of dry soil in SRI and conventional soils, respectively.

Nitrogen significantly increased the actinomycetes population irrespective of the stages. In summer season, the N₁₅₀ registered the highest mean actinomycetes population ranging from 6.3 to 11.3 x 10³ CFU g⁻¹ of dry soil and the lowest in N₀ ranging from 3.6 to 7.4 x 10³ CFU g⁻¹ of dry soil. Similar trend was also registered in *kharif* season. In both seasons, panicle initiation stage recorded the highest actinomycetes population and lowest in tillering. The interaction effect was non-significant.

4.1.4.4. Methanogens

Conventional system of rice cultivation registered significantly higher methanogen population than SRI in both the seasons (Table 4.20). In conventional system, the methanogen population ranged from 7.4 to 19.8 and 8.7 to 20.6 x 10⁴ CFU g⁻¹ of dry soil during summer and *kharif* seasons, respectively. The lowest methanogen population was observed in SRI soils, which ranged from 6.7 to 16.8 and 8.0 to 17.6 x 10⁴ CFU g⁻¹ of dry soil during summer and *kharif* seasons, respectively.

Incremental levels of nitrogen addition significantly increased the methanogens in both the seasons. The highest methanogens (8.2 to 19.8, 9.5 to 20.6 x 10⁴ CFU g⁻¹ of dry soil) was observed in N₁₅₀ and the lowest in N₀ (6.7 to 13.0, 8.0 to 13.8 x 10⁴ CFU g⁻¹ of dry soil) during summer and *kharif* seasons, respectively.

At flowering stage, methanogens was highest and the lowest was observed at maturity stage. The interaction effect was significant at all stages except during harvest in both summer and *kharif* seasons.

Table 4.19. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil actinomycetes population ($\times 10^3$ CFU g⁻¹ of dry soil)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	3.2	5.8	3.9	2.9	2.6	4.3	3.1	2.8
	N ₅₀	4.2	7.5	5.1	3.8	3.4	5.6	4	3.2
	N ₁₀₀	4.8	8.2	6.9	5.7	3.9	6.1	5.4	4.8
	N ₁₅₀	5.8	9.8	7.6	6.2	4.7	7.3	6	5.2
	N ₀	3.9	9.1	6.4	4.6	3.2	6.8	5	3.9
S ₂	N ₅₀	5.4	10.2	7.7	6.0	4.4	7.6	6.1	5
	N ₁₀₀	6.4	12.2	9.5	7.4	5.2	9.1	7.5	6.2
	N ₁₅₀	6.9	12.9	9.9	8.0	5.6	9.9	7.9	6.7
Mean	S ₁	4.5	7.8	5.9	4.7	3.7	5.8	4.6	4.0
	S ₂	5.7	11.1	8.4	6.5	4.6	8.4	6.6	5.5
	N ₀	3.6	7.4	5.1	3.8	2.9	5.6	4.1	3.4
	N ₅₀	4.8	8.8	6.4	4.9	3.9	6.6	5.1	4.1
	N ₁₀₀	5.6	10.2	8.2	6.5	4.6	7.6	6.5	5.5
	N ₁₅₀	6.3	11.3	8.8	7.1	5.2	8.6	7.0	6.0
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.11	0.34**	0.20	0.65**	0.08	0.28**	0.12	0.39*
	N	0.14	0.31**	0.27	0.58*	0.12	0.26**	0.16	0.34*
	S at N	0.21	NS	0.39	NS	0.17	NS	0.24	NS
	N at S	0.21	NS	0.39	NS	0.17	NS	0.23	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.20. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil methanogen population ($\times 10^4$ CFU g⁻¹ of dry soil)

Treatments		Summer (2011)				Kharif (2011)										
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest							
S ₁	N ₀	9.5	10.6	13.0	7.4	10.4	11.7	13.8	8.7							
	N ₅₀	13.4	16.8	17.2	7.9	14.3	17.9	18	9.2							
	N ₁₀₀	14.0	18.4	19.2	8.0	14.9	19.5	20	9.3							
	N ₁₅₀	14.5	18.8	19.8	8.6	15.4	19.9	20.6	9.9							
S ₂	N ₀	8.7	9.8	12.6	6.7	9.6	10.9	13.4	8							
	N ₅₀	9.1	13.1	15.5	7.5	10	14.2	16.3	8.8							
	N ₁₀₀	9.4	13.4	16.3	8.0	10.3	14.5	17.1	9.3							
	N ₁₅₀	10.4	14.2	16.8	8.2	11.3	15.3	17.6	9.5							
Mean	S ₁	12.9	16.2	17.3	8.0	13.8	17.3	18.1	9.3							
	S ₂	9.4	12.6	15.3	7.6	10.3	13.7	16.1	8.9							
	N ₀	9.1	10.2	12.8	7.1	10.0	11.3	13.6	8.4							
	N ₅₀	11.3	15.0	16.4	7.7	12.2	16.1	17.2	9.0							
	N ₁₀₀	11.7	15.9	17.8	8.0	12.6	17.0	18.6	9.3							
	N ₁₅₀	12.5	16.5	18.3	8.4	13.4	17.6	19.1	9.7							
		SEd	CD	SEd	CD	SEd	CD	SEd	CD							
S	0.20	0.64**	0.27	0.87**	0.30	0.98**	0.14	NS	0.21	0.69**	0.29	0.93**	0.32	1.02**	0.16	NS
N	0.34	0.71**	0.43	0.91**	0.48	1.02**	0.23	0.48**	0.36	0.77**	0.46	0.98**	0.51	1.07**	0.27	0.56**
S at N	0.46	1.07**	0.59	1.39**	0.67	1.56*	0.31	NS	0.49	1.15**	0.64	1.49**	0.70	NS	0.36	NS
N at S	0.48	1.01**	0.61	1.29**	0.68	1.44*	0.32	NS	0.52	1.09**	0.66	1.39**	0.72	NS	0.38	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

4.1.4.5. Methanotrophs

SRI soils had significantly recorded higher methanotrophs than conventional system in both seasons (Table 4.21). The highest methanotrophs was observed in SRI ranging from 15 to 40, 12 to 36 x 10³ CFU g⁻¹ of dry soil and the lowest in conventional system ranging from 9 to 34, 7 to 30 x 10³ CFU g⁻¹ of dry soil during summer and *kharif* seasons, respectively.

Incremental levels of nitrogen addition linearly decreased the methanotrophs in both SRI and conventional soils irrespective of growth stages. N₀ recorded the highest methanotrophs ranging from 20 to 40 and 16 to 36 x 10³ CFU g⁻¹ of dry soil during summer and *kharif* seasons, respectively. The lowest methanotrophs ranging from 9 to 28 and 7 to 25 x 10³ CFU g⁻¹ of dry soil was observed in N₁₅₀ during summer and *kharif* seasons, respectively.

Among the stages, the highest methanotrophs population was observed at harvest and the lowest at tillering stage in both the seasons. The interaction effect was significant at tillering stages in summer and in case of *kharif* season at tillering and panicle initiation stages.

4.1.4.6. Sulphate reducing bacteria

A marked variation in the population of sulphate reducers were observed in both SRI and conventional systems (Table 4.22). The population of sulphate reducing bacteria was significantly higher in conventional system than SRI at the tillering stage in both seasons. At tillering, conventional system recorded higher mean values of 9.4 and 10.4 x 10² CFU g⁻¹ of dry soil than SRI with the lowest mean values of 8.3 and 9.2 x 10² CFU g⁻¹ of dry soil during summer and *kharif* seasons, respectively. SRI registered higher population of sulphate reducers in comparison to conventional system at panicle initiation, flowering and harvest stages, but the values were significant only at harvest in both seasons.

Application of nitrogen fertilizers decreased the population of sulphate reducers at all stages, but the population was significant only at panicle initiation and flowering stages in both seasons. In summer, N₀ recorded significantly highest population of sulphate reducing bacteria with the mean values of 7.9 and 6.5 x 10² CFU g⁻¹ of dry soil and the lowest in N₁₅₀ with the mean values of 7.2 and 5.7 x 10² CFU g⁻¹ of dry soil. Similar trend of response was observed during *kharif*. The interaction effect between the systems of cultivation and nitrogen levels was non-significant.

Table 4.21. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil methanotrophs population ($\times 10^3$ CFU g⁻¹ of dry soil)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	20	23	27	34	16	18	24	30
	N ₅₀	14	17	21	30	11	13	18	27
	N ₁₀₀	12	17	18	28	9.4	13	16	25
	N ₁₅₀	9	14	17	25	7.4	11	15	22
S ₂	N ₀	30	32	32	40	24	25	28	36
	N ₅₀	22	26	26	35	17	20	23	31
	N ₁₀₀	19	24	24	31	15	19	21	28
	N ₁₅₀	15	21	19	28	12	15	17	25
Mean	S ₁	13.9	17.6	20.8	29.1	11.0	13.8	18.3	26.0
	S ₂	21.6	25.7	25.4	33.6	17.0	19.8	22.3	30.0
	N ₀	25.4	27.5	29.6	37.0	20.0	21.5	26.0	33.0
	N ₅₀	17.8	21.1	23.4	32.5	14.0	16.5	20.5	29.0
	N ₁₀₀	15.5	20.5	21.1	29.7	12.2	16.0	18.5	26.5
	N ₁₅₀	12.3	17.5	18.2	26.3	9.7	13.0	16.0	23.5
	S	SEd 0.32 CD 1.04**	SEd 0.40 CD 1.29**	SEd 0.40 CD 1.29**	SEd 0.55 CD 1.76**	SEd 0.25 CD 0.82**	SEd 0.31 CD 1.00**	SEd 0.35 CD 1.13**	SEd 0.49 CD 1.57**
	N	0.54 1.14**	0.64 1.35**	0.69 1.45**	0.93 1.96**	0.42 0.90**	0.50 1.05**	0.60 1.27**	0.83 1.75**
	S at N	0.74 1.71**	0.89 NS	0.94 NS	1.27 NS	0.58 1.35**	0.68 1.60*	0.82 NS	1.13 NS
	N at S	0.76 1.61**	0.91 NS	0.98 NS	1.32 NS	0.60 1.27**	0.70 1.48*	0.86 NS	1.17 NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.22. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil sulphate reducing bacteria ($\times 10^2$ CFU g⁻¹ of dry soil)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	9.9	7.7	6.4	5.0	11.0	8.1	7.4	5.1
	N ₅₀	9.2	7.6	6.1	5.0	10.2	8.0	7.1	5.0
	N ₁₀₀	9.5	7.3	6.0	5.2	10.5	7.6	7.0	5.3
	N ₁₅₀	9.0	7.2	5.6	5.1	10.0	7.6	6.5	5.6
	N ₀	8.5	8.2	6.7	5.8	9.4	8.6	7.8	5.9
S ₂	N ₅₀	8.3	8.1	6.3	5.6	9.2	8.5	7.3	5.5
	N ₁₀₀	8.2	7.4	6.5	5.4	9.2	7.8	7.6	5.6
	N ₁₅₀	8.1	7.1	5.8	5.3	9.0	7.5	6.8	5.8
Mean	S ₁	9.4	7.5	6.0	5.1	10.4	7.8	7.0	5.3
	S ₂	8.3	7.7	6.3	5.5	9.2	8.1	7.4	5.7
	N ₀	9.2	7.9	6.5	5.4	10.2	8.4	7.6	5.5
	N ₅₀	8.7	7.8	6.2	5.3	9.7	8.3	7.2	5.3
	N ₁₀₀	8.8	7.4	6.3	5.3	9.9	7.7	7.3	5.5
	N ₁₅₀	8.6	7.2	5.7	5.2	9.5	7.6	6.7	5.7
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.14	0.46**	0.13	NS	0.16	0.51**	0.14	NS
	N	0.26	NS	0.22	0.47**	0.29	NS	0.23	0.49**
	S at N	0.35	NS	0.30	NS	0.39	NS	0.32	NS
	N at S	0.37	NS	0.31	NS	0.41	NS	0.30	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

With regard to stages, the highest population of sulphate reducers was observed at tillering stage and thereafter the population declined gradually with the advancement of growth stages.

4.1.4.7. Total anaerobes

Conventional soils had significantly higher population of total anaerobes than SRI irrespective of stages of observation or seasons (Table 4.23). In conventional system, total anaerobes as observed at various stages ranged from 6.3×10^6 CFU g⁻¹ of dry soil at harvest to 19.9×10^6 CFU g⁻¹ of dry soil at the flowering stage in summer. Similar trend was observed during *kharif* season.

Addition of nitrogen significantly increased the load of total anaerobes at all stages of crop growth in both seasons. Highest population of total anaerobes was observed in N₁₅₀ which ranged from 7.0 to 19.9, 7.8 to 20.3 $\times 10^6$ CFU g⁻¹ of dry soil during summer and *kharif* seasons, respectively. Control (N₀) registered lowest population of total anaerobes which ranged from 6.3 to 13.0, 7.0 to 13.4 $\times 10^6$ CFU g⁻¹ of dry soil during summers and *kharif* seasons, respectively.

Population of total anaerobes showed a conspicuous increase till flowering stage and declined thereafter. The interaction effect between the systems of cultivation and nitrogen levels was significant at all stages except harvest in both seasons.

4.1.4.8. Dehydrogenase activity

The dehydrogenase activity was consistently higher in SRI soils than conventional system in both seasons (Table 4.24). SRI recorded significantly higher dehydrogenase activities in the range of 12.1 to 33.7 and 8.4 to 30.2 $\mu\text{g TPF g}^{-1}$ of soil hr⁻¹ while lower values (11.2 to 30.5 and 7.5 to 27.0 $\mu\text{g TPF g}^{-1}$ of soil hr⁻¹) were observed in conventional system during summer and *kharif* seasons, respectively.

Nitrogen fertilized soils had significantly the highest dehydrogenase activity regardless of crop growth stages or systems of cultivation. N₁₅₀ registered the highest dehydrogenase activity and the lowest in control (N₀) in both seasons. Among the stages, the highest and lowest dehydrogenase activities were observed at panicle initiation and tillering stages, respectively. The interaction effect was significant only at the harvest stage in both the seasons.

Table 4.23. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil total anaerobes ($\times 10^6$ CFU g⁻¹ of dry soil)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	10.0	12.3	13.0	6.3	12.2	12.9	13.4	7.0
	N ₅₀	13.2	17.2	19.5	7.0	15.0	18.1	19.9	7.8
	N ₁₀₀	13.9	18.8	19.5	7.5	15.7	19.8	19.9	8.3
	N ₁₅₀	14.1	19.6	19.9	7.9	16.0	20.5	20.3	8.8
S ₂	N ₀	9.9	11.2	12.3	6.3	11.2	11.8	12.6	7.0
	N ₅₀	10.4	13.8	12.4	6.7	11.8	14.5	12.7	7.4
	N ₁₀₀	11.0	14.0	14.8	6.8	12.5	14.7	15.1	7.7
	N ₁₅₀	11.4	14.3	15.5	7.0	12.9	15.0	15.8	7.8
Mean	S ₁	12.8	17.0	18.0	7.2	14.7	17.8	18.4	8.0
	S ₂	10.6	13.3	13.8	6.7	12.1	14.0	14.1	7.5
	N ₀	9.9	11.7	12.7	6.3	11.7	12.4	13.0	7.0
	N ₅₀	11.8	15.5	16.0	6.8	13.4	16.3	16.3	7.6
	N ₁₀₀	12.5	16.4	17.2	7.1	14.1	17.3	17.5	8.0
	N ₁₅₀	12.7	16.9	17.7	7.5	14.5	17.8	18.1	8.3
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.21	0.68**	0.24	0.90**	0.24	0.76**	0.29	0.93**
	N	0.35	0.74**	0.45	0.96**	0.40	0.85**	0.49	1.03**
	S at N	0.48	1.11**	0.62	1.45**	0.55	1.27*	0.67	1.55**
	N at S	0.49	1.04**	0.64	1.36**	0.57	1.20*	0.69	1.46**

*P≤0.05, **P ≤ 0.01, NS - Non significant

Table 4.24. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil dehydrogenase activity ($\mu\text{g TPF g}^{-1}$ of soil hr^{-1})

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	11.2	16.9	16.1	12.9	7.5	13.4	12.3	9.8
	N ₅₀	13.2	18.5	18.7	13.4	9.5	15	14.9	10.3
	N ₁₀₀	18.6	23.2	22.9	20.4	14.9	19.7	19.1	17.3
	N ₁₅₀	23.9	30.5	27.7	23.8	20.2	27.0	23.9	20.7
S ₂	N ₀	12.1	18.4	18.2	13.4	8.4	14.9	14.4	10.3
	N ₅₀	13.8	19.1	19.9	15.1	10.1	15.6	16.1	11.9
	N ₁₀₀	20.7	25.7	23.9	22.0	17	22.2	20.1	13.3
	N ₁₅₀	26.4	33.7	30.7	27.8	22.7	30.2	26.9	22.6
Mean	S ₁	16.7	22.3	21.4	17.6	13.0	18.8	17.6	14.5
	S ₂	18.3	24.2	23.2	19.6	14.6	20.7	19.4	14.5
	N ₀	11.7	17.7	17.2	13.2	8.0	14.2	13.4	10.1
	N ₅₀	13.5	18.8	19.3	14.3	9.8	15.3	15.5	11.1
	N ₁₀₀	19.7	24.5	23.4	21.2	16.0	21.0	19.6	15.3
	N ₁₅₀	25.2	32.1	29.2	25.8	21.5	28.6	25.4	21.7
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.37	1.19*	0.48	1.54*	0.31	0.98*	0.39	1.24*
	N	0.54	1.13**	0.70	1.48**	0.43	0.90**	0.56	1.17**
	S at N	0.75	NS	0.99	NS	0.61	NS	0.78	NS
	N at S	0.76	NS	1.00	NS	0.61	NS	0.79	NS

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

4.1.4.9. Phosphatase activity

SRI soils had higher phosphatase activity than conventional system, but the values were significant at tillering, panicle initiation, harvest stages during summer and in case of *kharif* season only at panicle initiation and harvest stages (Table 4.25). In SRI, the phosphatase activity ranged from 11.2 to 25.2 $\mu\text{g P-NPP g}^{-1}$ of soil hr^{-1} and 9.4 to 23.4 $\mu\text{g P-NPP g}^{-1}$ of soil hr^{-1} during summer and *kharif* seasons, respectively. The lowest phosphatase activity in conventional system ranged from 9.0 to 21.5 $\mu\text{g P-NPP g}^{-1}$ of soil hr^{-1} and 7.2 to 19.7 $\mu\text{g P-NPP g}^{-1}$ of soil hr^{-1} during summer and *kharif* seasons, respectively.

Nitrogen fertilized soils registered significantly highest phosphatase activity only at harvest stage in both seasons. The mean values are 23.4 and 10.1 $\mu\text{g P-NPP g}^{-1}$ of soil hr^{-1} in N_{150} and N_0 treatments, respectively during summer season. In *kharif* season, the mean values are 21.6 and 8.3 $\mu\text{g P-NPP g}^{-1}$ of soil hr^{-1} in N_{150} and N_0 treatments, respectively.

Among the stages, highest phosphatase activity was observed at harvest for SRI and in case of conventional system at tillering stage in both seasons. The interaction effect was significant only at harvest stage in summer season.

4.1.4.10. Urease activity

The urease activity did not significantly differ between the two systems of rice cultivation, but the values were consistently higher in SRI soils than conventional system in both seasons (Table 4.26).

Incremental levels of nitrogen addition linearly increased the urease activity regardless of the stages or seasons. N_{150} significantly recorded highest urease activity and the lowest activity in control (N_0). In summer season, the urease activity ranged from 18.3 to 22.5 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil hr^{-1} and 9.4 to 13.5 $\mu\text{g NH}_4\text{-N g}^{-1}$ of soil hr^{-1} in N_{150} and N_0 , respectively. Similar was the case observed during *kharif* season.

Among the stages, flowering recorded highest urease activity and the lowest was observed at harvest stage in both seasons. The interaction effect was non-significant.

Table 4.25. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil phosphatase activity (µg P- NPP g⁻¹ of soil hr⁻¹)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	15.2	13.3	12.1	9.0	13.5	11.6	10.7	7.2
	N ₅₀	15.3	13.2	12.1	12.5	13.6	11.5	10.6	10.7
	N ₁₀₀	15.7	13.4	12.2	14.8	14.0	11.7	10.7	15.7
	N ₁₅₀	15.6	13.6	12.0	21.5	14.1	11.9	10.5	19.7
S ₂	N ₀	16.5	14.1	12.0	11.2	14.3	12.4	10.5	9.4
	N ₅₀	16.6	14.3	12.1	13.6	14.2	12.6	10.6	11.8
	N ₁₀₀	16.5	14.2	12.3	20.0	14.6	12.5	10.8	18.2
	N ₁₅₀	16.7	14.3	12.1	25.2	14.8	12.6	11.0	23.4
Mean	S ₁	15.5	13.4	12.1	14.5	13.8	11.7	10.6	13.3
	S ₂	16.6	14.2	12.1	17.5	14.5	12.5	10.7	15.7
	N ₀	15.9	13.7	12.1	10.1	13.9	12.0	10.6	8.3
	N ₅₀	16.0	13.8	12.1	13.1	13.9	12.1	10.6	11.3
	N ₁₀₀	16.1	13.8	12.3	17.4	14.3	12.1	10.8	17.0
	N ₁₅₀	16.2	14.0	12.1	23.4	14.5	12.3	10.8	21.6
	S	SEd 0.29	CD 0.93*	SEd 0.21	CD NS	SEd 0.25	CD NS	SEd 0.18	CD NS
	N	0.47	NS	0.36	NS	0.41	NS	0.31	NS
	S at N	0.65	NS	0.49	NS	0.57	NS	0.43	NS
	N at S	0.67	NS	0.51	NS	0.59	NS	0.45	NS
	S	SEd 0.35	CD 1.13**	SEd 0.35	CD 1.13**	SEd 0.22	CD 0.70*	SEd 0.32	CD 1.03**
	N	0.49	NS	0.49	NS	0.36	NS	0.45	NS
	S at N	0.69	NS	0.69	NS	0.49	NS	0.64	NS
	N at S	0.69	NS	0.69	NS	0.50	NS	0.63	NS

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.26. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on soil urease activity ($\mu\text{g NH}_4\text{-N g}^{-1}$ of soil hr^{-1})

Treatments	Summer (2011)				Kharif (2011)			
	Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S₁	N ₀	9.4	11.4	13.3	9.7	12.0	13.1	11.2
	N ₅₀	14.2	16.3	19.3	13.9	16.8	18.0	15.4
	N ₁₀₀	16.3	17.8	20.6	17.0	18.9	19.5	18.5
	N ₁₅₀	20.0	21.3	21.5	18.3	22.6	23.0	19.8
S₂	N ₀	9.6	12.8	13.5	9.5	12.2	14.5	11.0
	N ₅₀	14.8	15.9	18.6	14.3	17.4	17.6	15.8
	N ₁₀₀	17.2	18.8	21.4	17.5	19.8	20.5	19.0
	N ₁₅₀	20.1	21.2	22.5	19.5	22.7	22.9	21.6
Mean	S ₁	15.0	16.7	18.7	14.7	17.6	18.4	16.2
	S ₂	15.4	17.2	19.0	15.2	18.0	18.9	16.9
	N ₀	9.5	12.1	13.4	9.6	12.1	13.8	11.1
	N ₅₀	14.5	16.1	19.0	14.1	17.1	17.8	15.6
	N ₁₀₀	16.8	18.3	21.0	17.3	19.4	20.0	18.8
	N ₁₅₀	20.1	21.3	22.0	18.9	22.7	23.0	20.7
		SEd	CD	SEd	CD	SEd	CD	SEd
	S	0.31	NS	0.35	NS	0.36	NS	0.32
	N	0.45	0.96**	0.50	1.06**	0.53	1.12**	0.49
	S at N	0.64	NS	0.71	NS	0.74	NS	0.68
	N at S	0.64	NS	0.71	NS	0.75	NS	0.69
		SEd	CD	SEd	CD	SEd	CD	SEd
	S	0.31	NS	0.35	NS	0.36	NS	0.32
	N	0.45	0.96**	0.50	1.06**	0.53	1.12**	0.49
	S at N	0.64	NS	0.71	NS	0.74	NS	0.68
	N at S	0.64	NS	0.71	NS	0.75	NS	0.69

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

4.1.5. Influence on carbon dioxide emissions

4.1.5.1. Carbon dioxide emission rate

Carbon dioxide (CO₂) emission rate was significantly higher in SRI than conventional system in both the seasons (Table 4.27). In summer, the CO₂ emission rate ranged from 3.26 to 15.48 and 1.79 to 8.50 mg m⁻² hr⁻¹ in SRI and conventional systems, respectively. During *kharif*, the CO₂ emission rate ranged from 1.58 to 11.04 and 1.34 to 4.85 mg m⁻² hr⁻¹ in SRI and conventional systems, respectively. In SRI, the CO₂ emission rate was significantly increased by 82.2 and 116.4 per cent, 58.3 and 29.7 per cent, 53.7 and 8.3 per cent, 17.5 and 12.5 per cent over the conventional system at tillering, panicle initiation, flowering and harvest stages during summer and *kharif* seasons, respectively.

Nitrogen addition significantly increased the CO₂ emissions at all the stages of observation during both seasons. N₁₅₀ registered increased CO₂ emissions by 27.3, 32.2, 36.1 and 39.0 per cent over the control (N₀) at tillering, panicle initiation, flowering and harvest stages, respectively during summer. A similar trend of response was observed during *kharif*. Among the stages, the highest CO₂ emission rate was recorded at the tillering and the lowest at panicle initiation stage during summer season. In case of *kharif* season, the lowest emission rate was recorded at flowering stage.

The interaction effect was significant only in *kharif* season at all stages except flowering.

4.1.5.2. Total CO₂ emission

The average daily CO₂ emission was higher in SRI than conventional system in both seasons (Table 4.28). In SRI, the average daily emission ranged from 1.73 to 2.19 and 0.93 to 1.51 kg ha⁻¹ d⁻¹ during summer and *kharif*, respectively. Conventional system registered the average daily emission ranging from 0.97 to 1.34 kg ha⁻¹ d⁻¹ and 0.57 to 0.86 kg ha⁻¹ d⁻¹ during summer and *kharif*, respectively. Total CO₂ emission was higher in SRI to the tune of 78.2 and 77.5 per cent over the conventional system during summer and *kharif*.

Nitrogen fertilization increased the average daily CO₂ emissions and the trend was similar in both seasons. N₁₅₀ registered the highest total CO₂ emission to tune of 13.2 and 18.4 per cent over control (N₀) during summer and *kharif* seasons, respectively.

** $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	6.44	1.85	1.79	4.36	3.31	1.83	1.34	2.26
	N ₅₀	7.53	2.33	2.50	5.39	3.98	2.51	1.83	3.10
	N ₁₀₀	8.12	2.59	2.65	5.80	4.47	2.70	1.97	3.33
	N ₁₅₀	8.50	2.64	2.82	6.36	4.85	2.82	2.06	3.47
	N ₀	12.40	3.26	3.31	5.53	6.53	2.55	1.58	2.12
S ₂	N ₅₀	13.09	3.60	3.63	6.08	8.25	2.94	1.86	2.45
	N ₁₀₀	14.81	3.93	3.96	6.74	10.11	3.40	2.10	2.83
	N ₁₅₀	15.48	4.09	4.11	7.41	11.04	3.87	2.26	3.22
Mean	S ₁	7.65	2.35	2.44	5.48	4.15	2.46	1.80	3.04
	S ₂	13.94	3.72	3.75	6.44	8.98	3.19	1.95	2.66
	N ₀	9.42	2.55	2.55	4.95	4.92	2.19	1.46	2.19
	N ₅₀	10.31	2.97	3.07	5.74	6.12	2.73	1.85	2.77
	N ₁₀₀	11.47	3.26	3.30	6.27	7.29	3.05	2.04	3.08
	N ₁₅₀	11.99	3.37	3.47	6.88	7.94	3.34	2.16	3.35
S	S	0.241	0.769**	0.068	0.217**	0.155	0.493**	0.037	0.167*
	N	0.318	0.670**	0.090	0.189**	0.199	0.418**	0.055	0.179*
	S at N	0.459	NS	0.130	NS	0.289	0.697**	0.077	0.271*
	N at S	0.451	NS	0.128	NS	0.281	0.592**	0.078	0.254*

Table 4.28. Seasonal and daily variations in carbon dioxide emission

Treatments		Total CO ₂ flux (kg ha ⁻¹)		Average daily emission (kg ha ⁻¹ d ⁻¹)	
		Summer	Kharif	Summer	Kharif
S ₁	N ₀	90.8	56.8	0.97	0.57
	N ₅₀	110.1	73.3	1.17	0.74
	N ₁₀₀	119.0	80.5	1.27	0.81
	N ₁₅₀	125.9	85.4	1.34	0.86
S ₂	N ₀	175.1	98.7	1.73	0.93
	N ₅₀	187.6	121.0	1.86	1.14
	N ₁₀₀	210.1	145.3	2.08	1.37
	N ₁₅₀	221.4	160.4	2.19	1.51

Overall the data suggest that the SRI registered an increase in the total CO₂ emissions by 78.2 and 77.5 per cent over conventional system during summer and *kharif*, respectively. The summer recorded the total CO₂ emissions of 51 per cent in comparison to *kharif* season.

4.1.6. Influence on methane emissions

4.1.6.1. Effect of cono-weeding on methane emission

Cono-weeding operations significantly decreased the methane emission in SRI soils during *kharif* season (Table 4.29). The methane estimation before and after cono-weeding operations revealed that four times of cono-weeding operations contributed for a significant decrease in methane emissions to the tune of 19.0, 14.5, 3.4 and 15.2 per cent over the control plots (before cono-weeding). In case of conventional system, where cono-weeding operation was not performed, an increasing trend in the methane emissions was observed during the same period of observation.

During the initial phase of methane measurements (before first cono-weeding), the average methane emission was higher in SRI (0.79 mg m⁻² hr⁻¹) than conventional (0.72 mg m⁻² hr⁻¹). In the next phase of observations i.e., when second to fourth conoweeding operations were undertaken in SRI, the conventional system recorded the significantly higher methane emission than SRI. Nitrogen fertilization significantly increased the methane emission except during the first phase of observation (before and after first cono-weeding).

The interaction effect was significant during the respective sampling period's viz., after second cono-weeding, before and after third cono-weeding, before and after fourth cono-weeding.

4.1.6.2. Methane emission rate

Methane emission rate was significantly higher in conventional system than SRI throughout the crop growth (Table 4.30). In conventional system, the methane emission rate ranged from 0.51 to 4.87 and 0.67 to 5.80 mg m⁻² hr⁻¹ during summer and *kharif* seasons, respectively. Methane emission rate in SRI ranged from 0.47 to 3.49 and 0.60 to 3.97 mg m⁻² hr⁻¹ in summer and *kharif*, respectively. The conventional system recorded

Table 4.29. Influence of cono-weeding on methane emission rate ($\text{mg m}^{-2} \text{ hr}^{-1}$) in SRI (S_2) system of rice cultivation during *kharif* season (2011)

Treatments		1 st Conoweeding		2 nd Conoweeding		3 rd Conoweeding		4 th Conoweeding	
		Before	After	Before	After	Before	After	Before	After
S ₂	N ₀	0.80	0.64	0.66	0.55	0.57	0.55	0.72	0.64
	N ₅₀	0.78	0.67	0.68	0.60	0.58	0.58	0.80	0.68
	N ₁₀₀	0.78	0.61	0.70	0.60	0.56	0.54	1.05	0.85
	N ₁₅₀	0.81	0.65	0.73	0.61	0.60	0.58	1.09	0.93
Comparison with conventional system (S ₁) of rice cultivation without cono-weeding									
S ₁	N ₀	0.69	0.78	0.96	0.98	1.06	1.15	1.21	1.20
	N ₅₀	0.70	0.80	1.08	1.20	1.15	1.32	1.40	1.64
	N ₁₀₀	0.75	0.79	1.13	1.48	1.52	1.70	1.76	1.95
	N ₁₅₀	0.72	0.80	1.20	1.83	1.96	1.90	1.95	2.18
Mean	S ₂	0.79	0.64	0.69	0.59	0.58	0.56	0.92	0.78
	S ₁	0.72	0.79	1.09	1.37	1.42	1.52	1.58	1.74
	N ₀	0.75	0.71	0.81	0.77	0.82	0.85	0.97	0.92
	N ₅₀	0.74	0.74	0.88	0.90	0.87	0.95	1.10	1.16
	N ₁₀₀	0.77	0.70	0.92	1.04	1.04	1.12	1.41	1.40
	N ₁₅₀	0.77	0.73	0.97	1.22	1.28	1.24	1.52	1.56
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.015	0.048*	0.013	0.043*	0.016	0.053**	0.017	0.056*
	N	NS	NS	NS	NS	0.036	0.076***	0.043	0.090*
	S at N	NS	NS	NS	NS	NS	NS	0.055	0.123*
	N at S	NS	NS	NS	NS	NS	NS	0.060	0.127*

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.30. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on methane emission rate (mg m⁻² hr⁻¹)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest
S ₁	N ₀	1.08	2.16	3.43	0.51	1.21	2.51	4.08	0.67
	N ₅₀	1.25	2.49	4.02	0.64	1.40	2.92	4.74	0.85
	N ₁₀₀	1.57	3.55	4.53	0.75	1.76	4.08	5.25	0.99
	N ₁₅₀	1.74	3.67	4.87	0.81	1.95	4.33	5.80	1.07
S ₂	N ₀	0.64	1.34	2.41	0.47	0.72	1.43	2.54	0.60
	N ₅₀	0.71	1.84	2.96	0.58	0.86	1.96	3.12	0.73
	N ₁₀₀	0.93	1.98	3.30	0.71	1.05	2.11	3.69	0.91
	N ₁₅₀	0.97	2.07	3.49	0.74	1.10	2.38	3.97	0.94
Mean	S ₁	1.41	2.97	4.21	0.68	1.58	3.46	4.97	0.89
	S ₂	0.81	1.81	3.04	0.63	0.93	1.97	3.33	0.80
	N ₀	0.86	1.75	2.92	0.49	0.97	1.97	3.31	0.63
	N ₅₀	0.98	2.17	3.49	0.61	1.13	2.44	3.93	0.79
	N ₁₀₀	1.25	2.77	3.91	0.73	1.41	3.10	4.47	0.95
	N ₁₅₀	1.35	2.87	4.18	0.77	1.53	3.35	4.88	1.01
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.018	0.059**	0.043	0.139**	0.022	0.071**	0.048	0.155**
	N	0.035	0.074**	0.075	0.157**	0.040	0.084**	0.087	0.183**
	S at N	0.047	0.107**	0.101	0.234**	0.054	0.123**	0.117	0.269**
	N at S	0.050	0.105**	0.106	0.223**	0.057	0.119**	0.123	0.259**

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

an increase in the methane emission rate by 74.1 and 69.9 per cent, 64.1 and 75.6 per cent, 38.5 and 49.2 per cent, 7.9 and 11.3 per cent over the SRI at tillering, panicle initiation, flowering and maturity stages during summer and *kharif* seasons, respectively.

Incremental levels of nitrogen addition significantly increased the methane emissions in both the seasons. Methane emission rate was the highest when nitrogen was applied @ 150 kg ha⁻¹ and the lowest in control (without nitrogen addition). In N₁₅₀, methane emission rate was increased by 57.0 and 57.7 per cent, 64 and 70.1 per cent, 43.2 and 47.4 per cent, 57.1 and 60.3 per cent over the control (N₀) at tillering, panicle initiation, flowering and maturity stages during summer and *kharif*, respectively.

Among stages, the flowering recorded the highest methane emission rate and the lowest at maturity stage regardless of the systems of cultivation or nitrogen fertilization. In both the seasons, the interaction effect was significant only at tillering and panicle initiation stages.

4.1.6.3. Total Methane emission

The average daily methane emission rate was higher in conventional and the values ranged from 0.36 to 0.58 kg ha⁻¹ d⁻¹ and 0.43 to 0.68 kg ha⁻¹ d⁻¹ during summer and *kharif*, respectively (Table 4.31). In SRI, average daily methane emission ranged from 0.24 to 0.37 kg ha⁻¹ d⁻¹ and 0.27 to 0.42 kg ha⁻¹ d⁻¹ during summer and *kharif*, respectively.

SRI had significantly recorded the lowest total methane emission and the values ranged from 24.4 to 36.9 kg ha⁻¹ and 28.3 to 45.0 kg ha⁻¹ during summer and *kharif* seasons, respectively. Conventional system contributed for higher total methane emissions and it ranged from 33.9 to 54.1 kg ha⁻¹ and 42.1 to 67.8 kg ha⁻¹ during summer and *kharif* seasons, respectively.

Nitrogen fertilization significantly contributed for increased methane emissions in terms of average daily emission as well as total methane emission. N₁₅₀ registered the highest total methane emission to tune of 34.6 and 36.5 per cent over control (N₀) during summer and *kharif*, respectively.

Overall, the data unequivocally demonstrated that the conventional system increased the total methane emissions by 40.3 and 47.1 per cent over SRI during summer

Table 4.31. Seasonal and daily variations in methane emission

Treatments		*Total methane emission (kg ha ⁻¹)		Average daily emission (kg ha ⁻¹ d ⁻¹)	
		Summer	Kharif	Summer	Kharif
S ₁	N ₀	33.9	42.1	0.36	0.43
	N ₅₀	39.5	49.3	0.42	0.50
	N ₁₀₀	50.9	62.6	0.54	0.63
	N ₁₅₀	54.1	67.8	0.58	0.68
S ₂	N ₀	24.4	28.3	0.24	0.27
	N ₅₀	30.6	35.9	0.30	0.34
	N ₁₀₀	35.3	41.6	0.35	0.39
	N ₁₅₀	36.9	45.0	0.37	0.42

and *kharif* seasons, respectively. The *kharif* season recorded an increase in the total methane emissions by 21.9 per cent over the summer.

4.1.7. Influence on growth and yield attributes

4.1.7.1. Plant height

The plant height was slightly taller in conventional system than SRI, however it was non-significant in both seasons (Table 4.32). Nitrogen fertilization significantly increased the plant height regardless of stages of observation. N₁₅₀ recorded the highest plant height and the lowest in control (N₀). The mean values at maturity stage were 87.8 cm and 78.9 cm, 89.6 cm and 82.0 cm in N₁₅₀ and N₀, respectively during summer and *kharif*, respectively. The interaction effect was non-significant.

4.1.7.2. Leaf Area Index (LAI)

Leaf Area Index (LAI) was significantly higher in conventional system than SRI irrespective of stages of observation or seasons (Table 4.33). The highest LAI was observed in conventional that ranged from 1.70 to 5.40, 1.75 to 6.43 and the lowest in SRI that ranged from 0.43 to 2.27, 0.45 to 2.49 during summer and *kharif*, respectively.

Application of nitrogen significantly increased the LAI in both the systems of rice cultivation. N₁₅₀ recorded significantly highest LAI and the mean values are 1.96, 2.60, 3.83 and 2.68 at tillering, panicle initiation, flowering and maturity stages, respectively during summer season. The lowest LAI was registered in control (N₀) and the mean values are 1.06, 1.78, 2.75 and 1.79 at tillering, panicle initiation, flowering and maturity stages, respectively during summer season. A similar trend was observed during *kharif* season. Among the stages, the highest and lowest LAI were recorded at flowering and tillering stages, respectively in both the seasons. The interaction effect was significant.

4.1.7.3. Number of Tillers per hill

The SRI had significantly recorded highest number of tillers per hill than conventional during both the seasons (Table 4.34). The mean values were 8.3, 9.7, 18.0 and 16.7 in SRI and in conventional system, the values were 7.8, 9.0, 11.4 and 10.9 at tillering, panicle initiation, flowering and maturity stages, respectively during summer season. A similar trend was observed during *kharif* season.

Table 4.32. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on plant height (cm)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Maturity	Tillering	Panicle Initiation	Flowering	Maturity
S₁	N₀	35.0	45.4	77.8	79.4	38.0	47.8	78.3	83.0
	N₅₀	36.3	47.8	81.0	82.7	41.7	50.3	79.2	84.0
	N₁₀₀	36.9	49.3	83.7	85.3	42.3	51.9	83.5	88.5
	N₁₅₀	40.6	51.2	85.3	87.0	44.0	53.9	85.0	90.1
S₂	N₀	32.1	43.9	76.9	78.4	35.6	46.2	76.4	81.0
	N₅₀	35.6	45.5	81.5	83.2	36.8	47.9	78.3	83.0
	N₁₀₀	36.7	47.6	83.6	85.2	38.9	50.1	83.0	88.0
	N₁₅₀	37.3	50.4	86.8	88.5	41.6	53.0	84.1	89.1
Mean	S₁	37.2	48.4	82.0	83.6	41.5	51.0	81.5	86.4
	S₂	35.4	46.8	82.2	83.8	38.2	49.3	80.5	85.3
	N₀	33.6	44.7	77.3	78.9	36.8	47.0	77.4	82.0
	N₅₀	35.9	46.6	81.3	82.9	39.3	49.1	78.8	83.5
	N₁₀₀	36.8	48.5	83.6	85.3	40.6	51.0	83.3	88.2
	N₁₅₀	39.0	50.8	86.0	87.8	42.8	53.5	84.6	89.6
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.66	NS	1.48	1.51	0.71	0.90	1.45	1.54
	N	1.08	2.27**	2.43	2.48	1.19	1.49	2.40	2.54
	S at N	1.48	NS	3.33	3.39	1.62	2.03	3.28	3.48
	N at S	1.52	NS	3.44	3.51	1.68	2.11	3.40	3.60

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.33. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on Leaf Area Index (LAI)

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Maturity	Tillering	Panicle Initiation	Flowering	Maturity
S ₁	N ₀	1.70	2.90	3.80	2.89	1.75	3.11	3.99	3.36
	N ₅₀	2.66	3.36	4.25	3.46	2.80	3.86	4.67	4.17
	N ₁₀₀	2.95	3.80	5.00	3.92	3.10	4.37	5.66	4.72
	N ₁₅₀	3.20	4.18	5.40	4.30	3.37	4.80	6.43	5.18
S ₂	N ₀	0.43	0.66	1.70	0.68	0.45	0.76	1.87	0.82
	N ₅₀	0.47	0.75	1.89	0.80	0.47	0.86	2.08	0.93
	N ₁₀₀	0.56	0.84	2.01	0.89	0.59	0.96	2.21	1.04
	N ₁₅₀	0.71	1.03	2.27	1.06	0.83	1.18	2.49	1.27
Mean	S ₁	2.63	3.56	4.61	3.64	2.76	4.04	5.19	4.36
	S ₂	0.54	0.82	1.97	0.86	0.59	0.94	2.16	1.02
	N ₀	1.06	1.78	2.75	1.79	1.10	1.94	2.93	2.09
	N ₅₀	1.57	2.05	3.07	2.13	1.64	2.36	3.38	2.55
	N ₁₀₀	1.75	2.32	3.51	2.40	1.85	2.67	3.94	2.88
	N ₁₅₀	1.96	2.60	3.83	2.68	2.10	2.99	4.46	3.23
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.009	0.029***	0.010	0.033***	0.009	0.031***	0.015	0.049***
	N	0.019	0.040***	0.022	0.047***	0.019	0.041***	0.028	0.060***
	S at N	0.025	0.057***	0.036	0.082*	0.026	0.058*	0.038	0.087***
	N at S	0.027	0.057***	0.032	0.067*	0.028	0.058*	0.040	0.085***

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.34. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on number of tillers per hill

Treatments		Summer (2011)				Kharif (2011)			
		Tillering	Panicle Initiation	Flowering	Maturity	Tillering	Panicle Initiation	Flowering	Maturity
S₁	N₀	5.9	6.8	9.6	9.3	6.1	8.6	10.3	9.7
	N₅₀	6.8	7.8	11.0	10.6	8.8	8.8	10.9	10.5
	N₁₀₀	8.9	10.2	11.5	11.1	9.1	9.4	12.0	11.5
	N₁₅₀	9.6	11.0	13.3	12.8	9.7	10.3	12.7	12.1
S₂	N₀	7.0	8.1	15.0	13.9	9.2	13.7	18.8	14.1
	N₅₀	7.4	8.5	16.8	15.6	9.5	14.0	20.7	16.2
	N₁₀₀	8.4	10.1	19.6	18.2	10.1	15.1	21.2	18.8
	N₁₅₀	10.5	12.1	20.7	19.2	12.3	16.4	22.4	21.5
Mean	S₁	7.8	9.0	11.4	10.9	8.4	9.3	11.5	10.9
	S₂	8.3	9.7	18.0	16.7	10.3	14.8	20.8	17.6
	N₀	6.5	7.5	12.3	11.6	7.7	11.2	14.6	11.9
	N₅₀	7.1	8.1	13.9	13.1	9.2	11.4	15.8	13.3
	N₁₀₀	8.7	10.2	15.6	14.6	9.6	12.3	16.6	15.1
	N₁₅₀	10.1	11.6	17.0	16.0	11.0	13.4	17.6	16.8
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.16	0.51*	0.32	0.94**	0.19	0.61**	0.36	1.14**
	N	0.24	0.50**	0.43	0.85**	0.27	0.57**	0.47	0.99**
	S at N	0.33	0.79*	0.61	1.38*	0.38	0.91**	0.68	NS
	N at S	0.34	0.71*	0.61	1.20*	0.39	0.81**	0.67	NS

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Incremental doses of nitrogen addition significantly increased the number of tillers per hill in both the seasons. The highest number of tillers per hill ranged from 9.6 to 20.7 in N_{150} and the lowest was recorded in N_0 that ranged from 5.9 to 15.0 during summer season. Similar was the trend during *kharif* season. The interaction effect was significant at tillering, flowering and maturity stages during summer and at tillering and maturity stages in *kharif* season.

4.1.7.4. Productive tillers

The productive tillers per hill at the maturity stage were significantly higher in SRI than conventional system in both the seasons (Table 4.35). Higher number of productive tillers per hill (13.0 to 18.0) was observed in SRI and the lowest in conventional system (8.7 to 11.9) during summer. A similar trend of response was recorded in *kharif* season. But, in case of productive tillers per m^2 , the conventional system recorded significantly higher than SRI. The productive tillers per m^2 ranged from 433 to 596 in conventional and it ranged from 207 to 287 numbers of productive tillers per m^2 in SRI during summer. In *kharif* season, the mean values were 536 and 262 numbers of productive tillers per m^2 in conventional and SRI systems, respectively.

Nitrogen levels significantly increased the productive tillers in both the seasons. N_{150} recorded significantly highest number of productive tillers per hill and the lowest in control (N_0). In summer season, the mean values were 14.9 and 10.8 in N_{150} and N_0 , respectively. Similar was the trend of response observed during *kharif* season. The interaction effect was significant.

4.1.7.5. Root volume

SRI plants had recorded significantly larger root volume than conventional plants regardless of the stages of observation or seasons (Table 4.36). At harvest stage, the root volume ranged varied from 23.3 to 36.3 cc and 16.9 to 25.8 cc in SRI and conventional systems, respectively during summer season. Similar trend of response was recorded during *kharif* season.

Application of nitrogen significantly increased the root volume irrespective of the systems of rice cultivation or stages of observation. N_{150} recorded the largest root volume ranging from 25.8 to 36.3 cc and 27.7 to 39.8 cc during summer and *kharif* seasons,

Table 4.35. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on productive tillers at maturity stage

Treatments		Summer (2011)				Kharif (2011)			
		Productive tillers per hill		Productive tillers per m ²		Productive tillers per hill		Productive tillers per m ²	
S ₁	N ₀	8.7		433		9.3		467	
	N ₅₀	9.9		495		10.1		504	
	N ₁₀₀	10.4		519		11.0		551	
	N ₁₅₀	11.9		596		11.7		583	
S ₂	N ₀	13.0		207		13.5		217	
	N ₅₀	14.6		233		15.5		249	
	N ₁₀₀	17.0		272		18.1		289	
	N ₁₅₀	18.0		287		20.7		331	
Mean	S ₁	10.2		510.5		10.0		536.0	
	S ₂	15.6		249.8		16.2		262.3	
	N ₀	10.8		319.9		10.9		335.9	
	N ₅₀	12.2		363.9		12.2		382.1	
	N ₁₀₀	13.7		395.3		13.9		415.0	
	N ₁₅₀	14.9		441.6		15.4		463.7	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.28	0.88**	6.10	19.42**	0.29	0.92**	6.10	19.43**
	N	0.38	0.79**	12.50	26.27**	0.40	0.85**	13.01	27.35**
	S at N	0.54	1.29*	16.49	37.09*	0.57	1.37**	17.07	NS
	N at S	0.53	1.12*	17.68	37.16*	0.58	1.21**	18.41	NS

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.36. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on root volume (cc)

Treatments		Summer (2011)				Kharif (2011)						
		Tillering	Panicle Initiation	Flowering	Harvest	Tillering	Panicle Initiation	Flowering	Harvest			
S ₁	N ₀	8.2	18.0	19.5	16.9	9.2	19.2	20.5	17.1			
	N ₅₀	10.6	23.4	26.2	22.8	11.9	24.9	27.6	23.9			
	N ₁₀₀	12.5	25.5	27.6	24.1	14.1	27.1	29.1	25.7			
	N ₁₅₀	14.2	27.6	29.6	25.8	16	29.4	31.2	27.7			
S ₂	N ₀	10.0	23.5	26.8	23.3	11	25	28.2	24.7			
	N ₅₀	12.0	29.9	32.6	28.3	13.2	31.8	34.3	30.5			
	N ₁₀₀	15.4	33.2	36.8	32.0	16.9	35.3	38.7	34.7			
	N ₁₅₀	20.6	37.3	41.7	36.3	22.6	39.7	43.9	39.8			
Mean	S ₁	11.4	23.6	25.7	22.4	12.8	25.2	27.1	23.6			
	S ₂	14.5	31.0	34.5	30.0	15.9	33.0	36.3	32.4			
	N ₀	9.1	20.8	23.1	20.1	10.1	22.1	24.4	20.9			
	N ₅₀	11.3	26.6	29.4	25.6	12.6	28.4	31.0	27.2			
	N ₁₀₀	14.0	29.3	32.2	28.0	15.5	31.2	33.9	30.2			
	N ₁₅₀	17.4	32.5	35.7	31.0	19.3	34.6	37.6	33.8			
		SEd	CD	SEd	CD	SEd	CD	SEd	CD			
S	0.28	0.91**	0.53	1.71**	0.52	1.64**	0.32	1.02**	0.63	1.87**	0.55	1.77**
N	0.52	1.10**	1.09	2.29**	1.20	2.53**	0.58	1.22**	1.27	2.45**	1.14	2.39**
S at N	0.70	1.60**	1.44	NS	1.59	NS	0.78	1.79**	1.68	NS	1.50	NS
N at S	0.74	1.55**	1.54	NS	1.70	NS	0.82	1.73**	1.80	NS	1.61	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

respectively, at harvest stage. Relatively smallest root volume was observed in N_0 that ranged from 16.9 to 23.3 cc and 17.1 to 24.7 cc during summer and *kharif* seasons, respectively at harvest stage. The interaction effect was significant at tillering stage in both seasons.

4.1.7.6. Root biomass

The root biomass expressed as g hill⁻¹ was significantly higher in SRI plants than conventional system in both seasons (Table 4.37). At harvest stage, the root biomass ranged from 9.6 to 14.9 g hill⁻¹ and 4.1 to 8.2 g hill⁻¹ in SRI and conventional plants, respectively during summer season. Similar trend of response was registered during *kharif* season. However, on per hectare basis, the root biomass expressed as kg ha⁻¹ was significantly higher in conventional system than SRI in both seasons. At harvest, the root biomass on per hectare basis, varied from 2050 to 4100 kg ha⁻¹ and 1536 to 2384 kg ha⁻¹ in conventional and SRI systems, respectively during summer season. In *kharif* season, the mean values are 3637 and 2068 kg ha⁻¹ in conventional and SRI systems, respectively at harvest.

Addition of nitrogen significantly increased the root biomass in both seasons. In both conventional and SRI systems, N_{150} recorded significantly highest root biomass (8.2 and 14.9 g hill⁻¹) and lowest (4.1 and 9.6 g hill⁻¹) in control (N_0), respectively during summer season. The trend of response was similar during *kharif* season. On per hectare basis, the highest root biomass was observed in N_{150} and the mean values are 3242 and 3539 kg ha⁻¹ during summer and *kharif* seasons, respectively. The interaction effect was significant for root biomass expressed as kg ha⁻¹ in both seasons.

4.1.7.7. Yield parameters

The grain yield under conventional system was significantly higher than SRI in both seasons (Table 4.38). Grain yield ranged from 4265 to 5731 kg ha⁻¹ and 3930 to 5386 kg ha⁻¹ in conventional and SRI, respectively during summer season. In *kharif* season similar trend of response was recorded which ranged from 4425 to 5885 kg ha⁻¹ and 4052 to 5572 kg ha⁻¹ in conventional and SRI, respectively. In conventional system, the grain yield increased by 6.6 per cent and 6.8 per cent over the SRI during summer and *kharif* seasons, respectively.

Table 4.37. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on root biomass

Treatments		Summer (2011)				Kharif (2011)			
		Root biomass (g hill ⁻¹)		Root biomass (kg ha ⁻¹)		Root biomass (g hill ⁻¹)		Root biomass (kg ha ⁻¹)	
S ₁	N ₀	4.1		2050		4.9		2450	
	N ₅₀	5.4		2700		6.4		3200	
	N ₁₀₀	7.0		3500		8.7		4350	
	N ₁₅₀	8.2		4100		9.1		4550	
S ₂	N ₀	9.6		1536		10.1		1616	
	N ₅₀	11.3		1808		11.8		1888	
	N ₁₀₀	13.7		2192		14.0		2240	
	N ₁₅₀	14.9		2384		15.8		2528	
Mean	S ₁	6.2		3087		7.3		3637	
	S ₂	12.4		1980		12.9		2068	
	N ₀	6.9		1793		7.5		2033	
	N ₅₀	8.4		2254		9.1		2544	
	N ₁₀₀	10.4		2846		11.4		3295	
	N ₁₅₀	11.6		3242		12.5		3539	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.23	0.74**	49.2	156.6**	0.22	0.71**	52.8	168.3**
	N	0.30	0.63**	79.8	167.8**	0.28	0.58**	91.4	192.1**
	S at N	0.43	NS	109.4	253.9**	0.41	NS	123.8	284.7*
	N at S	0.42	NS	112.9	237.2**	0.39	NS	129.3	271.8*

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.38. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation and different levels of nitrogen on yield (kg ha⁻¹) and harvest index (HI)

Treatments		Summer (2011)					Kharif (2011)								
		Grain yield	Straw yield	Biological yield	Harvest Index (HI)		Grain yield	Straw yield	Biological yield	Harvest Index (HI)					
S ₁	N ₀	4265	6765	11030	0.39		4425	6974	11399	0.39					
	N ₅₀	4830	6990	11820	0.41		4988	7248	12236	0.41					
	N ₁₀₀	5140	7250	12390	0.41		5296	7605	12901	0.41					
	N ₁₅₀	5731	7714	13445	0.43		5885	7960	13845	0.43					
S ₂	N ₀	3930	5837	9767	0.40		4052	5870	9922	0.41					
	N ₅₀	4510	6287	10797	0.42		4624	6310	10934	0.42					
	N ₁₀₀	4790	6598	11388	0.42		4980	6759	11739	0.42					
	N ₁₅₀	5386	7196	12582	0.43		5572	7358	12930	0.43					
Mean	S ₁	4992	7180	12171	0.41		5149	7447	12595	0.41					
	S ₂	4654	6480	11134	0.42		4807	6574	11381	0.42					
	N ₀	4098	6301	10399	0.39		4239	6422	10661	0.40					
	N ₅₀	4670	6639	11309	0.41		4806	6779	11585	0.42					
	N ₁₀₀	4965	6924	11889	0.42		5138	7182	12320	0.42					
	N ₁₅₀	5559	7455	13014	0.43		5729	7659	13388	0.43					
		SEd	CD	SEd	CD		SEd	CD	SEd	CD					
	S	102.4	326.1 *	145.7	463.7*	0.009	NS	105.8	336.8*	150.4	478.6**	256.1	815.1*	0.009	NS
	N	187.8	394.6**	263.8	554.4**	0.014	NS	193.8	407.1**	270.8	568.9**	464.5	975.8**	0.015	NS
	S at N	251.8	NS	354.5	NS	0.019	NS	259.8	NS	364.2	NS	623.8	NS	0.020	NS
	N at S	265.6	NS	373.1	NS	0.020	NS	274.1	NS	383.0	NS	656.8	NS	0.021	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Nitrogen fertilization registered significantly highest grain yield in both the seasons. N_{150} recorded highest grain yield and the mean values are 5559 and 5729 kg ha⁻¹ during summer and *kharif* seasons, respectively. The lowest grain yield was observed in control (N_0) and the mean values are 4098 and 4239 kg ha⁻¹ during summer and *kharif* seasons, respectively. The interaction effect was absent.

The conventional system had recorded significantly higher straw yield in comparison to SRI during both seasons. In conventional system, the straw yield increased by 9.7 and 11.7 per cent over the SRI during summer and *kharif* seasons, respectively. Incremental levels of nitrogen addition recorded significantly highest straw yield. N_{150} registered mean highest straw yield (7455 and 6301 kg ha⁻¹) and the lowest in control (N_0) in conventional and SRI systems, respectively during summer season. A similar trend of response was observed during *kharif* season. The interaction effect was non-significant.

The biological yield was significantly higher in conventional system than SRI in both seasons. The mean values are 12,171 and 11,134 kg ha⁻¹ in conventional and SRI systems, respectively during summer season. Similar trend of observations were recorded in *kharif* season and the mean values are 12,595 and 11,381 kg ha⁻¹ in conventional and SRI systems, respectively.

Addition of nitrogen significantly increased the biological yield and the highest was observed in N_{150} with the mean values 13,014 and 13,388 kg ha⁻¹ in summer and *kharif* seasons, respectively. Lowest biological yield was observed in control (N_0) and the mean values are 10,399 and 10,661 in summer and *kharif* seasons, respectively. The interaction effect was non-significant.

Though slight variations in the harvest index were observed but, it was non-significant regardless of systems of cultivation or nitrogen levels in both the seasons. In general, the harvest index varied between 0.39 and 0.43 in both the seasons.

4.1.8. Influence on plant carbon and nitrogen

4.1.8.1. Carbon content

The carbon contents of roots, straw and grains ranged from 39.0 to 39.8 per cent, 34.1 to 34.8 per cent and 42.0 to 42.3 per cent, respectively in both SRI and conventional

systems during summer season but the values were non-significant (Table 4.39). Similar trend of response was observed during *kharif* season.

4.1.8.2. Nitrogen content

Systems of rice cultivation did not significantly influence the nitrogen contents of roots, straw and grains in both seasons (Table 4.40). During summer season, the nitrogen contents of roots, straw and grains varied from 0.58 to 0.70 per cent, 0.84 to 0.97 per cent and 1.19 to 1.34 per cent, respectively in both SRI and conventional systems. A similar trend of response was observed during *kharif* season.

Application of nitrogen significantly increased the nitrogen contents of roots, straw and grains in both seasons. N_{150} recorded significantly highest nitrogen contents in roots, straw and grains and the mean values were 0.67, 0.98 and 1.37 per cent, respectively during summer season. Lowest nitrogen contents in roots, straw and grains were observed in control (N_0) and the mean values are 0.58, 0.88 and 1.22 per cent, respectively during summer season. The trend during *kharif* season was similar to summer. The interaction effect was non-significant.

4.1.8.3. Nitrogen uptake

The conventional system had recorded significantly higher nitrogen uptake in roots, straw and grains than SRI in both the seasons (Table 4.41). In conventional system, the mean nitrogen uptake in roots, straw and grains were 19.6 and 22.4 kg ha⁻¹, 65.7 and 70.2 kg ha⁻¹, 63.8 and 67.1 kg ha⁻¹ in summer and *kharif* seasons, respectively. In SRI, the mean nitrogen uptake in roots, straw and grains were 13.1 and 13.4 kg ha⁻¹, 57.2 and 59.8 kg ha⁻¹, 60.0 and 63.2 kg ha⁻¹ recorded in summer and *kharif* seasons, respectively.

Nitrogen significantly increased the nitrogen uptake in roots, straw and grains. N_{150} recorded significantly highest mean nitrogen uptake (22.1, 70.5 and 74.2 kg ha⁻¹) while the lowest values (10.6, 53.3 and 49 kg ha⁻¹) were observed in control (N_0) in the roots, straw and grains, respectively during summer season. Similar trend of response was observed during *kharif* season. The interaction effect in roots was significant.

Table 4.39. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on carbon content (%) of rice roots, straw and grains

Treatments		Summer (2011)			Kharif (2011)		
		Roots	Straw	Grains	Roots	Straw	Grains
S₁	N₀	39.0	34.6	42.1	39.2	33.9	42.6
	N₅₀	39.0	34.8	42.3	39.6	34.3	42.7
	N₁₀₀	39.4	34.2	42.2	39.0	34.5	42.3
	N₁₅₀	39.6	34.4	42.3	39.2	34.2	42.1
S₂	N₀	39.3	34.5	42.2	39.5	34.0	42.6
	N₅₀	39.6	34.3	42.0	39.1	34.5	42.1
	N₁₀₀	39.8	34.1	42.1	39.7	34.3	42.6
	N₁₅₀	39.8	34.6	42.1	39.4	34.7	42.4
Mean	S₁	39.3	34.2	42.4	39.3	34.5	42.2
	S₂	39.4	34.4	42.4	39.6	34.4	42.1
	N₀	39.4	34.0	42.6	39.2	34.6	42.2
	N₅₀	39.4	34.4	42.4	39.3	34.6	42.2
	N₁₀₀	39.4	34.4	42.5	39.6	34.2	42.2
	N₁₅₀	39.3	34.5	42.3	39.7	34.5	42.2
		SEd	CD	SEd	CD	SEd	CD
	S	0.70	NS	0.75	NS	0.61	NS
	N	1.16	NS	1.25	NS	1.01	NS
	S at N	1.59	NS	1.71	NS	1.38	NS
	N at S	1.65	NS	1.77	NS	1.43	NS

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.40. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on nitrogen content (%) of rice roots, straw and grains

Treatments		Summer (2011)			Kharif (2011)		
		Roots	Straw	Grains	Roots	Straw	Grains
S₁	N₀	0.58	0.85	1.20	0.56	0.88	1.22
	N₅₀	0.62	0.89	1.26	0.60	0.92	1.29
	N₁₀₀	0.64	0.94	1.30	0.62	0.97	1.33
	N₁₅₀	0.67	0.97	1.33	0.65	1.00	1.36
S₂	N₀	0.60	0.84	1.19	0.59	0.87	1.21
	N₅₀	0.65	0.87	1.28	0.64	0.90	1.31
	N₁₀₀	0.68	0.89	1.32	0.67	0.92	1.35
	N₁₅₀	0.70	0.92	1.34	0.69	0.95	1.37
Mean	S₁	0.61	0.94	1.30	0.63	0.91	1.27
	S₂	0.65	0.91	1.31	0.66	0.88	1.28
	N₀	0.58	0.88	1.22	0.59	0.85	1.20
	N₅₀	0.62	0.91	1.30	0.64	0.88	1.27
	N₁₀₀	0.65	0.95	1.34	0.66	0.92	1.31
	N₁₅₀	0.67	0.98	1.37	0.69	0.95	1.34
	S	0.011	0.016	0.023	0.011	0.016	0.023
	N	0.018	0.026	0.037	0.018	0.027	0.038
	S at N	0.026	0.036	0.051	0.025	0.037	0.052
	N at S	0.027	0.037	0.053	0.026	0.038	0.054

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.41. Influence of Conventional (S₁) and SRI (S₂) systems of rice cultivation at different levels of nitrogen on nitrogen uptake (kg ha⁻¹) of rice roots, straw and grains

Treatments		Summer (2011)						Kharif (2011)					
		Roots		Straw		Grains		Roots		Straw		Grains	
S ₁	N ₀	11.9		57.5		51.2		13.8		61.1		54.2	
	N ₅₀	16.7		62.2		60.9		19.2		66.4		64.1	
	N ₁₀₀	22.4		68.2		66.8		27.0		73.6		70.2	
	N ₁₅₀	27.5		74.8		76.2		29.6		79.5		79.8	
S ₂	N ₀	9.2		49.0		46.8		9.5		50.8		49.2	
	N ₅₀	11.8		54.7		57.7		12.0		56.5		60.4	
	N ₁₀₀	14.9		58.7		63.2		14.9		62.0		67.1	
	N ₁₅₀	16.7		66.2		72.2		17.3		69.7		76.2	
Mean	S ₁	19.6		65.7		63.8		22.4		70.2		67.1	
	S ₂	13.1		57.2		60.0		13.4		59.8		63.2	
	N ₀	10.6		53.3		49.0		11.6		55.9		51.7	
	N ₅₀	14.2		58.5		59.3		15.6		61.5		62.2	
	N ₁₀₀	18.7		63.4		65.0		21.0		67.8		68.6	
	N ₁₅₀	22.1		70.5		74.2		23.5		74.6		78.0	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
	S	0.32	1.02**	1.10	3.51**	1.17	3.75*	0.35	1.12**	1.17	3.72**	1.24	3.95*
	N	0.51	1.08**	1.85	3.88**	1.85	3.89**	0.57	1.21**	1.96	4.13**	1.94	4.09**
	S at N	0.70	1.64**	2.52	NS	2.55	NS	0.78	1.82**	2.67	NS	2.68	NS
	N at S	0.72	1.53**	2.61	NS	2.61	NS	0.81	1.71**	2.78	NS	2.75	NS

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

4.1.9. Influence on carbon sequestration pattern

The carbon sequestration pattern of conventional and SRI systems was assessed in system perspective considering the carbon flow in above ground (carbon in plant biomass) and below ground (root carbon and soil organic carbon) within the two distinct systems of rice cultivation (Table 4.42 and 4.43). The data revealed that SRI had significantly lower above ground and below ground carbon stock to the level of 9.5 and 7.9 per cent, 10.0 per cent and 7.0 per cent, respectively during summer and *kharif* seasons in comparison to conventional system.

Incremental levels of nitrogen fertilization had increased both the above ground and below ground carbon stock but the increase was linear and conspicuous for above ground carbon stock, while such response was less pronounced in below ground carbon stock. Overall, the carbon sequestration in SRI was conspicuously lower in comparison to conventional system. Despite nitrogen fertilization had progressively increased the carbon accumulation in rice cultivation, the highest value was registered when nitrogen was fertilized @ 150 kg ha⁻¹ which was significantly higher than rest of fertilization.

4.1.10. Influence on soil carbon sequestration pattern

The carbon sequestration pattern of conventional and SRI systems of rice cultivation was assessed based on the active and passive pools of carbon in soils (Table 4.44). The active pool of carbon was consistently higher in SRI than conventional system. The increase in microbial biomass carbon in SRI was 13.3 per cent and 16.3 per cent in summer and *kharif*, respectively. The passive pools of carbon comprising of humic acid, fulvic acid and glomalin were estimated higher values for SRI cultivation to the tune of 5.9 per cent, 3.4 per cent and 89.2 per cent in comparison to conventional system during summer. Similar trend of response was observed in *kharif*. In contrast, the humin fraction of passive carbon pool was registered lower values in SRI (7.1 per cent) in comparison to conventional system. The soil carbon stock assessment has shown that SRI registered lower values by 5.6 per cent and 4.0 per cent in summer and *kharif* seasons, respectively in comparison to conventional system.

Table 4.42. Carbon sequestration pattern as influenced by Conventional (S_1) and SRI (S_2) systems of rice cultivation at different levels of nitrogen during summer (2011)

Treatments		Carbon Stock (kg ha ⁻¹)		
		Above ground carbon stock	Below ground carbon stock	Total Carbon Stock
S_1	N_0	4136	16887	21023
	N_{50}	4476	16947	21423
	N_{100}	4649	17209	21858
	N_{150}	5078	17261	22338
S_2	N_0	3672	15855	19527
	N_{50}	4051	15817	19867
	N_{100}	4267	15866	20132
	N_{150}	4757	15771	20528
Mean	S_1	4585	17076	21661
	S_2	4187	15827	20014
	N_0	3904	16371	20275
	N_{50}	4264	16382	20645
	N_{100}	4458	16538	20995
	N_{150}	4918	16516	21433

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.43. Carbon sequestration pattern as influenced by Conventional (S_1) and SRI (S_2) systems of rice cultivation at different levels of nitrogen during *kharif* (2011)

Treatments		Carbon Stock (kg ha ⁻¹)		
		Above ground carbon stock	Below ground carbon stock	Total Carbon Stock
S_1	N_0	4249	21455	25704
	N_{50}	4616	21529	26145
	N_{100}	4864	21789	26653
	N_{150}	5200	21749	26949
S_2	N_0	3722	20255	23977
	N_{50}	4124	20207	24330
	N_{100}	4440	20231	24671
	N_{150}	4916	20190	25105
Mean	S_1	4732	21631	26363
	S_2	4301	20221	24521
	N_0	3986	20855	24841
	N_{50}	4370	20868	25238
	N_{100}	4652	21010	25662
	N_{150}	5058	20970	26027

* $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.44. Influence of conventional and SRI systems on soil carbon sequestration pattern at harvest stage

Parameters	Summer (2011)		Kharif season (2011)	
	Conventional	SRI	Conventional	SRI
Active carbon pool				
Microbial Biomass Carbon (kg ha ⁻¹)	390	442	307	357
Passive carbon pools				
Humic acid (kg ha ⁻¹)	4652	4925	5014	5456
Fulvic acid (kg ha ⁻¹)	6321	6533	7243	7732
Humins (kg ha ⁻¹)	11803	11025	16412	15833
Glomalin (kg ha ⁻¹)	794	1502	486	1481
Soil Organic Carbon (%)	0.740	0.701	0.955	0.918
Soil Carbon Stock (kg ha⁻¹)	15873	15036	20198	19416

4.2. Laboratory experiment

4.2.1. Soil organic carbon dynamics

The moisture regime and nitrogen levels significantly influenced the soil organic carbon (SOC) dynamics (Table 4.45). Alternate wetting and drying with nitrogen significantly decreased the SOC during the incubation period and recorded the lowest SOC (6.08 per cent) at the end of incubation (63 days after incubation). Alternate wetting and drying with nitrogen decreased the SOC by 12.3 per cent over the initial SOC content followed by alternate wetting and drying without nitrogen (8.9 per cent), submergence with nitrogen (8.7 per cent) and submergence without nitrogen (7.7 per cent). The interaction effect was absent.

4.2.2. CO₂ evolution

Among the treatments, alternate wetting and drying with nitrogen recorded significantly highest CO₂ evolution throughout the incubation period (Table 4.46). The lowest CO₂ evolution was observed in submergence without nitrogen. During the 63 days of incubation, the mean values of CO₂ evolution are 391, 268, 234 and 199 mg kg⁻¹ in alternate wetting and drying with nitrogen, alternate wetting and drying without nitrogen, submergence with nitrogen and submergence without nitrogen. The interaction effect was significant.

4.3. Pot culture experiment under OTC conditions

4.3.1. Initial soil characteristics

The soil used in the pot experiment was sandy clay and belongs to Noyyal series. It is classified taxonomically as *Typic Ustochrept* according to USDA classification (Table 4.47). The results revealed that the soil was slightly alkaline (pH =8.21) with low soluble salts (EC = 0.35). The soil was high in organic carbon content (6.78 g kg⁻¹), low in available nitrogen (110.3 mg kg⁻¹), medium in available phosphorus and potassium (6.8 mg kg⁻¹ and 118.0 mg kg⁻¹), respectively. The microbial biomass carbon was 161 mg kg⁻¹ and microbial biomass nitrogen was observed as 14.9 mg kg⁻¹.

Table 4.45. Effect of moisture regime and nitrogen levels on the dynamics of soil organic carbon (g kg⁻¹)

Treatments	Incubation (days)										
	0 th	7 th	14 th	21 st	28 th	35 th	42 nd	49 th	56 th	63 rd	Mean
T ₁	6.94	6.87	6.76	6.67	6.60	6.53	6.44	6.40	6.35	6.32	6.59
T ₂	6.94	6.85	6.67	6.51	6.41	6.30	6.21	6.16	6.10	6.08	6.42
T ₃	6.94	6.89	6.80	6.71	6.66	6.59	6.53	6.48	6.44	6.40	6.64
T ₄	6.94	6.90	6.79	6.73	6.65	6.56	6.48	6.42	6.37	6.33	6.62
Mean	6.94	6.88	6.76	6.66	6.58	6.50	6.42	6.37	6.31	6.28	6.57
	SEd				CD						
T	0.021				0.041**						
D	0.033				0.065**						
T x D	0.066				NS						
T ₁ - Alternate Wetting & Drying without nitrogen											
T ₂ - Alternate Wetting & Drying with nitrogen											
T ₃ - Submergence without nitrogen											
T ₄ - Submergence with nitrogen											

*P≤0.05, ** P≤0.01, NS - Non significant

Table 4.46. Effect of moisture regime and nitrogen levels on soil CO₂ evolution (mg kg⁻¹)

Treatments	Incubation (days)									
	7 th	14 th	21 st	28 th	35 th	42 nd	49 th	56 th	63 rd	Mean
T ₁	257	403	330	311	298	292	197	183	140	268
T ₂	330	660	587	486	403	383	254	221	195	391
T ₃	183	331	257	228	220	198	129	137	110	199
T ₄	147	394	317	293	276	242	164	148	126	234
Mean	229	447	373	330	299	279	186	172	143	273
	SEd					CD				
T	1.0					1.9**				
D	1.5					2.9**				
T x D	3.0					5.9**				
T ₁ - Alternate Wetting & Drying without nitrogen										
T ₂ - Alternate Wetting & Drying with nitrogen										
T ₃ - Submergence without nitrogen										
T ₄ - Submergence with nitrogen										

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

Table 4.47. Initial soil characteristics of Open Top Chamber (OTC) experiment

S.No.	Parameters	Values*
1.	Soil Taxonomy	<i>Vertic Ustochrept</i>
2.	Soil series	Noyyal
a) Mechanical properties		
3.	Textural class	Sandy clay
4.	Sand (%)	43.6
5.	Silt (%)	9.1
6.	Clay (%)	45.2
b) Physico-chemical properties		
7.	pH	8.21
8.	EC (dSm ⁻¹)	0.35
c) Chemical properties		
9.	Organic carbon (g kg ⁻¹)	6.78
10.	Available Nitrogen (mg kg ⁻¹)	110.3
11.	Available Phosphorus (mg kg ⁻¹)	6.8
12.	Available Potassium (mg kg ⁻¹)	118.0
d) Biological properties		
13.	Microbial biomass carbon (mg kg ⁻¹)	161
14.	Microbial biomass nitrogen (mg kg ⁻¹)	14.9

* mean of four replications

4.3.2. Effect of elevated CO₂ on chemical properties

4.3.2.1. Soil organic carbon (SOC)

Elevated CO₂ levels did not significantly influence the soil organic carbon (SOC) content regardless of stages of observation (Table 4.48). At harvest, the mean SOC values were 6.52, 6.63 and 6.63 g kg⁻¹, respectively under 380, 550 and 750 μmol mol⁻¹ CO₂ concentrations.

Nitrogen fertilization significantly decreased the SOC at all levels of CO₂ concentrations. The highest mean SOC values were observed in N₀ (7.12, 6.96 and 6.74 g kg⁻¹) while the values were lowest in N₂₀₀ (7.00, 6.75 and 6.51 g kg⁻¹) at tillering, flowering and harvest stages, respectively. The SOC contents decreased gradually with the advancement of crop growth. The interaction effect was non-significant.

4.3.2.2. Dissolved organic carbon (DOC)

The dissolved organic carbon (DOC) was significantly highest under elevated levels of CO₂ (Table 4.48). Highest dissolved organic carbon (DOC) was observed under C₇₅₀ levels and the lowest under C_{amb} (ambient) conditions. DOC ranged from 533 to 722 mg L⁻¹ and 368 to 501 mg L⁻¹ in C₇₅₀ and C_{amb}, respectively.

Incremental levels of nitrogen addition significantly increased the DOC regardless of the stages of measurements. Highest DOC contents were recorded in N₂₀₀ and the mean values were 534, 621 and 507 mg L⁻¹ at tillering, flowering and harvest stages, respectively. Control (N₀) registered the lowest DOC contents and the mean values are 455, 528 and 436 mg L⁻¹ at tillering, flowering and harvest stages, respectively. Among the stages, flowering recorded the highest DOC contents and lowest at harvest stage. The interaction effect was non-significant.

4.3.2.3. Available nitrogen

The soil available nitrogen was significantly decreased under elevated CO₂ levels irrespective of the growth stages (Table 4.49). The highest mean soil available nitrogen values (119.8, 112.1 and 101.5 mg kg⁻¹) were observed under ambient (C_{amb}) while the lowest values (116.8, 109.6 and 98.2 mg kg⁻¹) under C₇₅₀ at tillering, flowering and harvest stages, respectively.

Treatments		SOC (g kg ⁻¹)			DOC (mg L ⁻¹)							
		Tillering	Flowering	Harvest	Tillering	Flowering	Harvest					
C _{amb}	N ₀	7.12	6.96	6.75	368	427	350					
	N ₁₅₀	7.05	6.80	6.61	418	485	397					
	N ₂₀₀	6.99	6.77	6.50	430	501	409					
C ₅₅₀	N ₀	7.13	6.97	6.73	464	538	440					
	N ₁₅₀	7.04	6.81	6.62	529	614	508					
	N ₂₀₀	7.00	6.75	6.53	548	639	523					
C ₇₅₀	N ₀	7.12	6.96	6.74	533	618	517					
	N ₁₅₀	7.06	6.84	6.63	607	703	573					
	N ₂₀₀	7.00	6.74	6.51	624	722	590					
Mean	C _{amb}	7.05	6.84	6.62	405	471	385					
	C ₅₅₀	7.06	6.84	6.63	514	597	490					
	C ₇₅₀	7.06	6.85	6.63	588	681	560					
	N ₀	7.12	6.96	6.74	455	528	436					
	N ₁₅₀	7.05	6.82	6.62	518	601	493					
	N ₂₀₀	7.00	6.75	6.51	534	621	507					
C	N	C x N	SEd	CD	SEd	CD	SEd	CD				
			0.057	NS	0.055	NS	4.1	8.4**	4.8	9.8		
			0.057	NS	0.055	0.113*	4.1	8.4**	4.8	9.8		
			0.098	NS	0.096	NS	7.2	NS	8.3	NS	6.8	NS

CO₂ levels : C_{amb} - 380 μ mol mol⁻¹ CO₂; C₅₅₀ - 550 μ mol mol⁻¹ CO₂; C₂ - 750 μ mol mol⁻¹ CO₂

Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹

CO₂ levels : C_{amb} - 380 μmol mol⁻¹ CO₂; C₅₅₀ - 550 μmol mol⁻¹ CO₂; C₂₋₇₅₀ μmol mol⁻¹ CO₂
 Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹
 *P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Treatments		Available Nitrogen (mg kg ⁻¹)			Available Phosphorus (mg kg ⁻¹)			Available Potassium (mg kg ⁻¹)		
		Tillering	Flowering	Harvest	Tillering	Flowering	Harvest	Tillering	Flowering	Harvest
C _{amb}	N ₀	106.9	98.9	89.3	11.6	10.7	9.8	131.2	120.7	115.9
	N ₁₅₀	122.4	115.1	103.6	10.0	9.4	8.6	127.1	116.9	111.2
	N ₂₀₀	130.1	122.3	111.6	9.0	8.6	7.8	123.0	113.2	107.2
C ₅₅₀	N ₀	107.2	100.8	90.7	11.0	10.2	9.3	129.8	119.4	114.6
	N ₁₅₀	120.3	113.0	101.7	9.4	8.9	8.0	124.9	114.9	109.8
	N ₂₀₀	127.8	120.1	107.0	8.6	8.0	7.3	121.0	111.3	105.0
C ₇₅₀	N ₀	107.8	102.3	92.1	10.7	9.8	8.6	128.0	117.8	112.5
	N ₁₅₀	117.1	109.2	98.3	8.9	8.3	7.4	121.3	111.6	107.1
	N ₂₀₀	125.5	117.4	104.2	8.0	7.1	6.5	120.0	109.0	104.6
Mean	C _{amb}	119.8	112.1	101.5	10.2	9.6	8.7	127.1	116.9	111.4
	C ₅₅₀	118.4	111.3	99.8	9.7	9.0	8.2	125.2	115.2	109.8
	C ₇₅₀	116.8	109.6	98.2	9.2	8.4	7.5	123.1	112.8	108.1
	N ₀	107.3	100.7	90.7	11.1	10.2	9.2	129.7	119.3	114.3
	N ₁₅₀	119.9	112.4	101.2	9.4	8.9	8.0	124.4	114.5	109.4
	N ₂₀₀	127.8	119.9	107.6	8.6	7.9	7.2	121.3	111.2	105.6
C	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
N	0.68	1.39*	0.64	1.30*	0.58	1.17**	0.07	0.13**	0.07	0.13**
C x N	0.68	1.39**	0.64	1.30**	0.58	1.17**	0.07	0.13**	0.07	0.13**
	0.18	NS	1.11	2.26*	1.00	2.03**	0.14	NS	0.12	NS
CO ₂ levels : C _{amb} - 380 μ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μ mol mol ⁻¹ CO ₂ ; C ₇₅₀ - 750 μ mol mol ⁻¹ CO ₂										
Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹										

CO ₂ levels	: C _{amb} - 380 μmol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μmol mol ⁻¹ CO ₂ ; C ₂ - 750 μmol mol ⁻¹ CO ₂
CO ₂ levels	: C _{amb} - 380 μmol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μmol mol ⁻¹ CO ₂ ; C ₂ - 750 μmol mol ⁻¹ CO ₂

CO₂ levels : C_{amb} – 500 μmol mol⁻¹ CO₂, C₅₅₀ – 550 μmol mol⁻¹ CO₂,
Nitrogen levels : N₀ – 0 kg N ha⁻¹, N₁₅₀ – 150 kg N ha⁻¹, N₂₀₀ – 200 kg N ha⁻¹

** $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Nitrogen fertilization significantly increased the soil available nitrogen regardless of CO₂ concentrations or stages of observation. The highest mean soil available nitrogen values (127.8, 119.9 and 107.6 mg kg⁻¹) were recorded in N₂₀₀ while the lowest values (107.3, 100.7 and 90.7 mg kg⁻¹) in N₀ at tillering, flowering and harvest stages, respectively. A decline in the available nitrogen status was observed with the progression of crop growth. The interaction effect was significant at flowering and harvest stages.

4.3.2.4. Available phosphorus

The elevated CO₂ levels significantly decreased the soil available phosphorus in comparison to ambient CO₂ concentrations irrespective of stages (Table 4.49). The highest mean soil available phosphorus values (10.2, 9.6 and 8.7 mg kg⁻¹) were observed in C_{amb} while the lowest values (9.2, 8.4 and 7.5 mg kg⁻¹) in C₇₅₀ at tillering, flowering and harvest stages, respectively.

Soil available phosphorus contents significantly decreased with increasing levels of nitrogen addition. The highest mean available phosphorus values (11.1, 10.2 and 9.2 mg kg⁻¹) were recorded in N₀ while the lowest values (8.6, 7.9 and 7.2 mg kg⁻¹) in N₂₀₀ at tillering, flowering and harvest stages, respectively. A gradual decline in the soil available phosphorus status was registered with the advancement of crop growth. The interaction effect was absent.

4.3.2.5. Available potassium

The soil available potassium was significantly decreased under elevated CO₂ concentrations irrespective of stages (Table 4.49). The highest mean soil available potassium values (127.1, 116.9 and 111.4 mg kg⁻¹) were observed under ambient (C_{amb}) while the lowest values (123.1, 112.8 and 108.1 mg kg⁻¹) under C₇₅₀ at tillering, flowering and harvest stages, respectively.

Nitrogen addition significantly decreased the soil available potassium regardless of CO₂ levels or stages of observation. N₀ recorded the lowest mean available potassium values (129.7, 119.3 and 114.3 mg kg⁻¹) while the lowest values (121.3, 111.2 and 105.6 mg kg⁻¹) in N₂₀₀ at tillering, flowering and harvest stages, respectively. A steep decline in the available potassium contents were observed with the advancement of crop growth. The interaction effect was non-significant.

4.3.3. Effect of elevated CO₂ on biological properties

4.3.3.1. Microbial biomass carbon

The microbial biomass carbon was significantly highest under elevated CO₂ levels (Table 4.50). C₇₅₀ registered the highest mean microbial biomass carbon values (284, 334 and 302 mg kg⁻¹ soil) while the lowest values (241, 285 and 254 mg kg⁻¹ soil) were observed under C_{amb} (ambient) at tillering, flowering and harvest stages, respectively.

Application of nitrogen, significantly increased the microbial biomass carbon under all levels of CO₂. The highest mean microbial biomass carbon values (301, 355 and 319 mg kg⁻¹) were recorded in N₂₀₀ and the lowest values (204, 239 and 215 mg kg⁻¹ soil) at tillering, flowering and harvest stages, respectively. Among the stages, flowering registered the highest microbial biomass carbon and the lowest at tillering stage under all levels of CO₂. The interaction effect was significant.

4.3.3.2. Microbial biomass nitrogen

Elevated CO₂ levels had significantly increased the soil microbial biomass nitrogen regardless of stages of observation (Table 4.50). The highest mean microbial biomass nitrogen values (26.4, 30.2 and 26.0 mg kg⁻¹ soil) were observed in C₇₅₀ while the lowest values (22.6, 25.7 and 22.1 mg kg⁻¹ soil) in C_{amb} (ambient) at tillering, flowering and harvest stages, respectively.

Incremental levels of nitrogen addition significantly increased the soil microbial biomass nitrogen at all CO₂ concentrations irrespective of stages. N₂₀₀ registered the highest mean microbial biomass nitrogen values (28.3, 32.3 and 27.9 mg kg⁻¹ soil) while the lowest values (18.8, 21.3 and 18.5 mg kg⁻¹ soil) were observed in control (N₀) conditions at tillering, flowering and harvest stages, respectively. Among the stages, flowering and tillering recorded the highest and lowest microbial biomass nitrogen, respectively. The interaction effect was significant.

4.3.3.3. Methanogens

Methanogen population was significantly highest under elevated CO₂ levels regardless of stages of observation (Table 4.51). C₇₅₀ had significantly highest methanogen population which ranged from 5.7 to 20.1 x 10⁴ CFU g⁻¹ of dry soil while the lowest

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Table 4.51. Effect of elevated CO₂ and nitrogen levels on soil methanogens and methanotrophs population

Treatments		Methanogens ($\times 10^4$ CFU g ⁻¹ of dry soil)			Methanotrophs ($\times 10^3$ CFU g ⁻¹ of dry soil)		
		Tillering	Flowering	Harvest	Tillering	Flowering	Harvest
C_{amb}	N₀	7.60	10.1	5.1	11.4	11.9	12.5
	N₁₅₀	11.80	15.1	8.2	7.3	7.7	7.9
	N₂₀₀	13.10	16.9	9.1	6.3	6.5	6.9
C₅₅₀	N₀	8.10	11.3	5.3	12.4	12.8	13.1
	N₁₅₀	13.30	17.6	8.6	8.6	9.0	9.5
	N₂₀₀	14.60	19.5	9.5	6.7	7.2	7.6
C₇₅₀	N₀	8.90	12.4	5.7	12.9	13.1	13.6
	N₁₅₀	14.20	18.9	9.0	10.2	10.7	11.0
	N₂₀₀	14.90	20.1	9.7	7.1	7.9	8.3
Mean	C_{amb}	10.8	14.0	7.5	8.4	8.7	9.1
	C₅₅₀	12.0	16.1	7.8	9.2	9.7	10.1
	C₇₅₀	12.7	17.1	8.1	10.1	10.5	11.0
	N₀	8.2	11.3	5.4	12.2	12.6	13.1
	N₁₅₀	13.1	17.2	8.6	8.7	9.1	9.5
	N₂₀₀	14.2	18.8	9.4	6.7	7.2	7.6
		SEd	CD	SEd	CD	SEd	CD
C	0.09	0.20**	0.13	0.27**	0.08	0.16**	0.12**
N	0.09	0.20**	0.13	0.27**	0.08	0.16**	0.12**
C x N	0.17	0.35*	0.23	0.46*	0.13	0.27**	0.21**
CO ₂ levels : C _{amb} - 380 μ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μ mol mol ⁻¹ CO ₂ ; C ₇₅₀ - 750 μ mol mol ⁻¹ CO ₂ Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹ *P ≤ 0.05, **P ≤ 0.01, NS - Non significant							

population ranged from 5.1 to 16.9×10^4 CFU g⁻¹ of dry soil under ambient (C_{amb}) concentration. At flowering, the mean values are 14.0, 16.1 and 17.1×10^4 CFU g⁻¹ of dry soil under 380, 550 and $750 \mu\text{mol mol}^{-1}$ CO₂ concentrations, respectively.

Nitrogen significantly increased the methanogen population irrespective of CO₂ levels or stages. The highest methanogen load (9.1 to 20.1×10^4 CFU g⁻¹ of dry soil) was observed in N_{200} while the lowest population (5.1 to 12.4×10^4 CFU g⁻¹ of dry soil) was recorded in N_0 . Among the stages, flowering registered the highest methanogen population and the lowest at harvest stage irrespective of CO₂ levels. The interaction effect was significant only at tillering and flowering stages.

4.3.3.4. Methanotrophs

Elevated CO₂ levels significantly increased the soil methanotrophs population irrespective of stages of observation (Table 4.51). The highest methanotrophs population ranging from 7.1 to 13.6×10^3 CFU g⁻¹ of dry soil was observed in C_{750} while the lowest population ranging from 6.3 to 12.5×10^3 CFU g⁻¹ of dry soil in ambient CO₂ concentration. At harvest, the mean values are 9.1, 10.1 and 11.0×10^3 CFU g⁻¹ of dry soil under 380, 550 and $750 \mu\text{mol mol}^{-1}$ CO₂ concentrations, respectively.

Incremental levels of nitrogen addition significantly decreased the methanotrophs population regardless of CO₂ concentrations or stages of observation. The highest mean values (12.2 , 12.6 and 13.1×10^3 CFU g⁻¹ of dry soil) were observed in N_0 while the lowest population (6.7 , 7.2 and 7.6×10^3 CFU g⁻¹ of dry soil) was recorded in N_{200} at tillering, flowering and harvest stages, respectively. Among the stages, harvest registered the highest methanotrophs population and the lowest at tillering stage irrespective of CO₂ levels. The interaction effect was significant.

4.3.4. Redox potential

Redox potential remained unaltered throughout the growth phase of rice crop regardless of CO₂ concentrations or nitrogen levels (Table 4.52). In general, the redox potential ranged between -276 mV and -281 mV and the values were non-significant. The interaction effect was obviously non-significant.

Table 4.52. Effect of elevated CO₂ and nitrogen levels on soil redox potential

Treatments		Redox potential (mV)					
		Tillering		Flowering		Harvest	
C _{amb}	N ₀	-277		-280		-279	
	N ₁₅₀	-277		-278		-277	
	N ₂₀₀	-276		-278		-280	
C ₅₅₀	N ₀	-277		-279		-279	
	N ₁₅₀	-277		-280		-277	
	N ₂₀₀	-278		-278		-277	
C ₇₅₀	N ₀	-277		-280		-278	
	N ₁₅₀	-276		-281		-279	
	N ₂₀₀	-276		-279		-278	
Mean	C _{amb}	-277		-279		-279	
	C ₅₅₀	-277		-279		-278	
	C ₇₅₀	-276		-280		-278	
	N ₀	-277		-280		-279	
	N ₁₅₀	-277		-280		-278	
	N ₂₀₀	-277		-278		-278	
		SEd	CD	SEd	CD	SEd	CD
C		1.7	NS	1.7	NS	1.7	NS
N		1.7	NS	1.7	NS	1.7	NS
C x N		3.0	NS	3.0	NS	3.0	NS
CO ₂ levels : C _{amb} - 380 μ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μ mol mol ⁻¹ CO ₂ ; C ₂ - 750 μ mol mol ⁻¹ CO ₂							
Nitrogen levels : N ₀ – 0 kg N ha ⁻¹ ; N ₁₅₀ – 150 kg N ha ⁻¹ ; N ₂₀₀ – 200 kg N ha ⁻¹							

**P* ≤ 0.05, ** *P* ≤ 0.01, NS - Non significant

4.3.5. Effect of elevated CO₂ on methane emissions

4.3.5.1. Methane emission rate

Elevated CO₂ levels significantly increased the methane emission rate irrespective of stages of observation (Table 4.53). CO₂ concentration @ 750 μ mol mol⁻¹ CO₂ recorded the highest methane emission rate (0.67 to 8.52 mg pot⁻¹d⁻¹) while the lowest rate (0.50 to 5.54 mg pot⁻¹d⁻¹) was observed under ambient concentration. The highest mean methane emission values (2.70, 7.67 and 0.88 mg pot⁻¹d⁻¹) in C₇₅₀ and the lowest values (1.87, 4.98 and 0.66 mg pot⁻¹d⁻¹) in C_{amb} were recorded at tillering, flowering and harvest stages, respectively. C₇₅₀ increased the methane emission rate by 44.4, 54.0 and 33.3 per cent over C_{amb} at tillering, flowering and harvest stages, respectively. Under C₅₅₀ levels, the methane emission rate increased by 27.8, 32.1 and 21.2 per cent over C_{amb} at tillering, flowering and harvest stages, respectively. The methane emission rate increased under C₇₅₀ to the tune of 13.0, 16.6 and 10 per cent over C₅₅₀ at tillering, flowering and harvest stages, respectively.

Addition of nitrogen significantly increased the methane emission rate regardless of CO₂ levels or stages of observation. The highest mean values (2.74, 7.15 and 0.98 mg pot⁻¹d⁻¹) and the lowest values (1.75, 5.30 and 0.59 mg pot⁻¹d⁻¹) were recorded in N₂₀₀ and N₀ at tillering, flowering and harvest stages, respectively. At flowering stage, the highest methane emission rate was recorded while harvest registered the lowest. The interaction effect was significant.

4.3.5.2. Average methane emission

The average methane emissions ranged from 2.02 to 2.85 mg pot⁻¹d⁻¹, 2.60 to 3.74 mg pot⁻¹d⁻¹ and 3.02 to 4.27 mg pot⁻¹d⁻¹ under 380, 550 and 750 μ mol mol⁻¹ CO₂, concentrations, respectively (Table 4.53). The mean values are 2.51, 3.26 and 7.75 mg pot⁻¹d⁻¹ under C_{amb}, C₅₅₀ and C₇₅₀ levels, respectively. With respect to nitrogen levels, the mean values are 2.55, 3.35 and 3.62 mg pot⁻¹d⁻¹ in N₀, N₁₅₀ and N₂₀₀, respectively.

4.3.5.3. Total Methane emission

The total methane emission was highest under 750 μ mol mol⁻¹ CO₂ followed by 550 μ mol mol⁻¹ CO₂ and the lowest under 380 μ mol mol⁻¹ CO₂ concentrations (ambient) (Table 4.53). Total methane emission ranged from 0.22 to 0.31 g pot⁻¹, 0.29 to 0.41 g pot⁻¹

Treatments		Methane emission (mg pot ⁻¹ d ⁻¹)			Average methane emission (mg pot ⁻¹ d ⁻¹)	Total Methane emission (g pot ⁻¹)
		Tillering	Flowering	Harvest		
Camb	N ₀	1.43	4.13	0.50	2.02	0.22
	N ₁₅₀	2.00	5.27	0.66	2.64	0.29
	N ₂₀₀	2.19	5.54	0.83	2.85	0.31
C ₅₅₀	N ₀	1.79	5.41	0.61	2.60	0.29
	N ₁₅₀	2.54	6.96	0.80	3.43	0.38
	N ₂₀₀	2.83	7.38	1.00	3.74	0.41
C ₇₅₀	N ₀	2.04	6.35	0.67	3.02	0.33
	N ₁₅₀	2.88	8.15	0.87	3.97	0.44
	N ₂₀₀	3.19	8.52	1.09	4.27	0.47
Mean	C _{amb}	1.87	4.98	0.66	2.51	0.28
	C ₅₅₀	2.39	6.58	0.80	3.26	0.36
	C ₇₅₀	2.70	7.67	0.88	3.75	0.41
	N ₀	1.75	5.30	0.59	2.55	0.28
	N ₁₅₀	2.47	6.79	0.78	3.35	0.37
	N ₂₀₀	2.74	7.15	0.98	3.62	0.40
C N C x N	SED	CD	SED	CD	SED	CD
	0.019	0.039**	0.053	0.108**	0.007	0.014**
	0.019	0.039**	0.053	0.108**	0.007	0.014**
	0.034	0.069**	0.093	0.118*	0.012	0.024*

CO₂ levels : C_{amb} - 380 μmol mol⁻¹ CO₂; C₅₅₀ - 550 μmol mol⁻¹ CO₂; C₂ - 750 μmol mol⁻¹ CO₂
Nitrogen levels : N₀ - 0 kg N ha⁻¹, N₁₅₀ - 150 kg N ha⁻¹, N₂₀₀ - 200 kg N ha⁻¹

** $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

and 0.33 to 0.47 g pot⁻¹ under C_{amb}, C₅₅₀ and C₇₅₀ levels, respectively. The highest mean value (0.41 g pot⁻¹) was observed in C₇₅₀ while the lowest value (0.28 g pot⁻¹) in C_{amb}. C₇₅₀ increased the total methane emission by 46.4 per cent and 13.9 per cent over the C_{amb} and C₅₅₀ levels, respectively. The increase in the total methane emission was to the tune of 28.6 per cent in C₅₅₀ level over the ambient CO₂ concentration.

Total methane emission increased with increasing doses of nitrogen fertilizers. N₂₀₀ increased the total methane emission by 42.9 per cent and 8.1 per cent over the N₀ and N₁₅₀ levels, respectively. The increase in the total methane emission was to the tune of 32.1 per cent in N₁₅₀ over the control (N₀).

4.3.6. Effect of elevated CO₂ levels on growth attributes

4.3.6.1. Plant height

The plants exposed to elevated CO₂ levels were tallest in comparison to ambient CO₂ concentrations (Table 4.54). The highest mean plant height (34.6, 63.8, 66.9 cm) was recorded under C₇₅₀ and relatively smallest (32.1, 57.3, 59.2 cm) in C_{amb} at tillering, flowering and harvest stages, respectively. Nitrogen fertilized plants were significantly tallest irrespective of CO₂ levels or stages. The highest mean values (36.0, 66.0 and 69.4 cm) were recorded in N₂₀₀ while the lowest values (29.6, 53.7 and 54.9 cm) in N₀ at tillering, flowering and harvest stages, respectively. The interaction effect was significant.

4.3.6.2. Number of tillers per hill

The maximum number of tillers hill⁻¹ was recorded under elevated CO₂ levels (Table 4.54). At harvest, the number of tillers hill⁻¹ ranged from 6.7 to 9.4, 7.8 to 12.0 and 8.1 to 13.7 were recorded under C_{amb}, C₅₅₀ and C₇₅₀, respectively. Nitrogen levels had registered significantly maximum number of tillers hill⁻¹. At harvest, the mean values for number of tillers hill⁻¹ were 7.5, 10.9 and 11.7 under N₀, N₁₅₀, N₂₀₀, respectively. The interaction effect was significant.

4.3.7. Effect of elevated CO₂ levels on rice physiology

4.3.7.1. Chlorophyll contents

Elevated CO₂ levels significantly influenced the chlorophyll contents of leaves irrespective of the stages of observations (Table 4.55). At tillering stage, the highest mean chlorophyll a, chlorophyll b and total chlorophyll (1.39, 0.59 and 2.50 mg g⁻¹, respectively)

Treatments		Plant height (cm)			Tiller number (tillers hill ⁻¹)			
		Tillering	Flowering	Harvest	Tillering	Flowering	Harvest	
C _{amb}	N ₀	29.3	53.0	53.6	3.7	6.8	6.7	
	N ₁₅₀	33.1	58.9	61.3	4.8	9.0	8.7	
	N ₂₀₀	33.9	60.0	62.8	5.2	10.0	9.4	
C ₅₅₀	N ₀	29.5	53.7	54.8	4.4	8.0	7.8	
	N ₁₅₀	36.4	67.0	71.0	6.3	11.7	11.3	
	N ₂₀₀	37.0	68.8	72.3	6.7	12.4	12.0	
C ₇₅₀	N ₀	29.9	54.4	56.3	4.6	8.5	8.1	
	N ₁₅₀	36.7	67.9	71.3	7.1	12.9	12.6	
	N ₂₀₀	37.2	69.2	73.0	7.7	14.3	13.7	
Mean	C _{amb}	32.1	57.3	59.2	4.6	8.6	8.3	
	C ₅₅₀	34.3	63.2	66.0	5.8	10.7	10.4	
	C ₇₅₀	34.6	63.8	66.9	6.5	11.9	11.5	
	N ₀	29.6	53.7	54.9	4.2	7.8	7.5	
	N ₁₅₀	35.4	64.6	67.9	6.0	11.2	10.9	
	N ₂₀₀	36.0	66.0	69.4	6.5	12.2	11.7	
C x N	SEd	CD	SEd	CD	SEd	CD	SEd	CD
	0.19	0.39**	0.36	0.72**	0.37	0.75**	0.06	0.12**
	0.19	0.39**	0.36	0.72**	0.37	0.75**	0.06	0.12**
CO ₂ levels	SEd	CD	SEd	CD	SEd	CD	SEd	CD
	0.34	0.68**	0.62	1.25**	0.64	1.31**	0.11	0.22**

CO₂ levels : C_{amb} - 380 μ mol mol⁻¹ CO₂; C₅₅₀ - 550 μ mol mol⁻¹ CO₂; C₇₅₀ - 750 μ mol mol⁻¹ CO₂
 Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹
 *P ≤ 0.05, **P ≤ 0.01, NS - Non significant

CO₂ levels : C_{amb} - 380 μmol mol⁻¹ CO₂; C₅₅₀ - 550 μmol mol⁻¹ CO₂; C₂₋₇₅₀ μmol mol⁻¹ CO₂
 Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹
 *P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.55. Effect of elevated CO₂ and nitrogen levels on chlorophyll a, chlorophyll b and total chlorophyll contents

Treatments	Chlorophyll 'a' content (mg g ⁻¹)			Chlorophyll 'b' content (mg g ⁻¹)			Total Chlorophyll content (mg g ⁻¹)		
	Tillering	Flowering	Harvest	Tillering	Flowering	Harvest	Tillering	Flowering	Harvest
C_{amb}	N₀	1.22	1.42	0.79	0.52	0.65	2.19	2.68	1.69
	N₁₅₀	1.35	1.83	1.15	0.58	0.83	2.43	3.46	2.46
	N₂₀₀	1.46	1.94	1.19	0.62	0.88	2.62	3.66	2.55
C₅₅₀	N₀	1.16	1.35	0.72	0.51	0.61	2.10	2.55	1.54
	N₁₅₀	1.43	1.70	1.08	0.60	0.76	2.56	3.20	2.31
	N₂₀₀	1.49	1.81	1.13	0.64	0.82	2.68	3.41	2.41
C₇₅₀	N₀	1.13	1.29	0.65	0.48	0.58	2.03	2.43	1.39
	N₁₅₀	1.51	1.64	1.04	0.63	0.75	2.70	3.11	2.21
	N₂₀₀	1.54	1.76	1.07	0.67	0.79	2.78	3.32	2.29
Mean	C_{amb}	1.34	1.73	1.05	0.57	0.79	2.41	3.27	2.23
	C₅₅₀	1.36	1.62	0.98	0.58	0.73	2.45	3.06	2.09
	C₇₅₀	1.39	1.56	0.92	0.59	0.71	2.50	2.95	1.96
	N₀	1.17	1.35	0.72	0.50	0.61	2.11	2.55	1.54
	N₁₅₀	1.43	1.72	1.09	0.60	0.78	2.56	3.26	2.33
	N₂₀₀	1.50	1.83	1.13	0.64	0.83	2.69	3.47	2.42
C x N	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd
	0.011	0.022*	0.013	0.027**	0.008	0.017**	0.005	0.010*	0.004
	0.011	0.022**	0.013	0.027**	0.008	0.017**	0.005	0.010**	0.004
C x N	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd
	0.019	0.039**	0.023	NS	0.014	NS	0.009	0.018**	0.011
	0.019	0.039**	0.023	NS	0.014	NS	0.009	0.018**	0.011
CO ₂ levels : C _{amb} - 380 µ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 µ mol mol ⁻¹ CO ₂ ; C ₇₅₀ - 750 µ mol mol ⁻¹ CO ₂									
Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹									
*P ≤ 0.05, **P ≤ 0.01, NS - Non significant									

were recorded in C_{750} , while the lowest values (1.34, 0.57 and 2.41 mg g^{-1} , respectively) in C_{amb} . The trend of response was reverse at flowering and harvest stages under the elevated CO_2 levels. The highest mean chlorophyll a, chlorophyll b and total chlorophyll (1.73, 0.79 and 3.27 mg g^{-1} , respectively) were recorded in C_{amb} , while the lowest values (1.56, 0.71 and 2.95 mg g^{-1} , respectively) in C_{750} at flowering stage.

Nitrogen addition had significantly increased the chlorophyll contents irrespective of CO_2 levels or stages of observation. At harvest stage, the mean chlorophyll a, chlorophyll b and total chlorophyll (1.13, 0.47 and 2.42 mg g^{-1} , respectively) were recorded in N_{200} , while the lowest values (0.72, 0.30 and 1.54 mg g^{-1} , respectively) in control (N_0).

Among the stages, flowering recorded the highest chlorophyll contents of leaves while the harvest stage registered the lowest. The interaction effect was significant for the parameters such as chlorophyll a, chlorophyll b and total chlorophyll at only tillering stage.

4.3.7.2. Leaf nitrogen content

The leaf nitrogen contents were significantly influenced by elevated CO_2 concentrations, but the values were significant only at flowering and harvest stages (Table 4.56). With reference to tillering stage, the leaf nitrogen contents were slightly highest under elevated CO_2 levels, however it was non-significant. The highest mean leaf nitrogen values (2.01 and 0.76 per cent) were observed under C_{amb} and the lowest values (1.82 and 0.67 per cent) under C_{750} at flowering and harvest stages, respectively.

The application of nitrogen fertilizers significantly increased the leaf nitrogen contents regardless of the CO_2 levels or stages. N_{200} registered the highest mean leaf nitrogen values (2.00, 2.12 and 0.84 per cent) while the lowest values (1.53, 1.58 and 0.52 per cent) were observed in control (N_0) at tillering, flowering and harvest stages, respectively. Among the stages, the flowering registered the highest leaf nitrogen and harvest stage recorded the lowest. The interaction effect was significant only at tillering stage.

4.3.7.3. Soluble proteins

The elevated CO_2 concentrations had recorded significantly highest soluble proteins, however the values were significant only at flowering and harvest stages (Table 4.56). In the case of tillering stage, the soluble proteins were numerically highest under elevated CO_2

Treatments		Leaf Nitrogen content (%)			Soluble protein content (mg g ⁻¹)							
		Tillering	Flowering	Harvest	Tillering	Flowering	Harvest					
C _{amb}	N ₀	1.62	1.65	0.57	7.5	13.6	4.2					
	N ₁₅₀	1.82	2.13	0.83	8.4	17.9	6.1					
	N ₂₀₀	1.96	2.25	0.89	9.0	18.7	6.5					
C ₅₅₀	N ₀	1.52	1.58	0.52	7.0	13.3	3.8					
	N ₁₅₀	1.91	1.97	0.78	8.8	16.5	5.7					
	N ₂₀₀	1.99	2.10	0.83	9.2	17.6	6.0					
C ₇₅₀	N ₀	1.46	1.52	0.47	6.7	12.8	3.4					
	N ₁₅₀	1.98	1.91	0.75	9.1	16.0	5.5					
	N ₂₀₀	2.05	2.02	0.79	9.4	17.0	5.8					
Mean	C _{amb}	1.80	2.01	0.76	8.3	16.7	5.6					
	C ₅₅₀	1.81	1.88	0.71	8.3	15.8	5.2					
	C ₇₅₀	1.83	1.82	0.67	8.4	15.3	4.9					
	N ₀	1.53	1.58	0.52	7.1	13.2	3.8					
	N ₁₅₀	1.90	2.00	0.79	8.8	16.8	5.7					
	N ₂₀₀	2.00	2.12	0.84	9.2	17.8	6.1					
C N C x N	SEd	CD	SEd	CD	SEd	CD	SEd	CD				
	0.015	NS	0.016	0.032**	0.006	0.012**	0.069	NS	0.131	0.267**	0.043	0.087***
	0.015	0.030**	0.016	0.032**	0.006	0.012**	0.069	0.139**	0.131	0.267**	0.043	0.087***
CO ₂ levels Nitrogen levels : *P ≤ 0.05, ** P ≤ 0.01, NS - Non significant	0.026	0.053**	0.027	NS	0.011	NS	0.119	0.242**	0.228	NS	0.075	NS
	CO ₂ levels : C _{amb} - 380 μ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μ mol mol ⁻¹ CO ₂ ; C ₇₅₀ - 750 μ mol mol ⁻¹ CO ₂											
	Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹											

CO₂ levels : C_{amb} - 380 μmol mol⁻¹ CO₂; C₅₅₀ - 550 μmol mol⁻¹ CO₂; C₂ - 750 μmol mol⁻¹ CO₂
 Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹
 *P ≤ 0.05, **P ≤ 0.01, NS - Non significant

levels, but it was non-significant. At flowering and harvest stages, the plants exposed to elevated CO₂ levels registered significantly lowest soluble proteins. The highest mean soluble proteins (16.7 and 5.6 mg g⁻¹) were observed under C_{amb} and the lowest values (15.3 and 4.9 mg g⁻¹) under C₇₅₀ at flowering and harvest stages, respectively. Incremental levels of nitrogen addition significantly increased the soluble proteins regardless of the CO₂ levels or stages. N₂₀₀ recorded the highest mean soluble proteins values (9.2, 17.8 and 6.1 mg g⁻¹) while the lowest values (7.1, 13.2 and 3.8 mg g⁻¹) were registered in control (N₀) at tillering, flowering and harvest stages, respectively. Among the stages, the flowering registered the highest soluble proteins and harvest stage recorded the lowest. The interaction effect was significant only at tillering stage.

4.3.8. Effect of elevated CO₂ levels on nitrogen assimilatory enzymes

4.3.8.1. Nitrate reductase

The plant exposed to elevated CO₂ levels did not exhibit any changes in the nitrate reductase activity in leaves in comparison to ambient at tillering stage (Table 4.57). Conversely, the nitrate reductase activities in leaves were significantly decreased under elevated CO₂ levels at flowering and maturity stages. The mean values were 0.66, 0.86 and 0.31 μ moles NO₂ g⁻¹ hr⁻¹ in C₇₅₀ and 0.65, 0.95 and 0.36 μ moles NO₂ g⁻¹ hr⁻¹ in C_{amb} at tillering, flowering and maturity stages, respectively.

Under elevated CO₂ levels, the nitrate reductase activities in roots were unaltered at tillering, significantly increased at flowering and decreased at maturity stage in comparison to plants grown under ambient CO₂ levels.

Nitrogen application significantly increased the nitrate reductase activities in leaves and roots irrespective of CO₂ levels or stages. At flowering stage, the mean values in leaves and roots are 1.01 and 0.63 μ moles NO₂ g⁻¹ hr⁻¹ in N₂₀₀ and 0.75 and 0.47 μ moles NO₂ g⁻¹ hr⁻¹ in control (N₀).

Among the stages, the highest nitrate reductase activities in leaves and roots were observed at flowering and the lowest at harvest. The interaction effect was significant only at tillering stage in leaves and roots.

Treatments		Leaves ($\mu\text{moles NO}_2 \text{ g}^{-1} \text{ hr}^{-1}$)			Roots ($\mu\text{moles NO}_2 \text{ g}^{-1} \text{ hr}^{-1}$)								
		Tillering	Flowering	Harvest	Tillering	Flowering	Harvest						
C _{amb}	N ₀	0.58	0.78	0.27	0.23	0.45	0.10						
	N ₁₅₀	0.66	1.01	0.39	0.26	0.57	0.15						
	N ₂₀₀	0.71	1.07	0.41	0.28	0.60	0.16						
C ₅₅₀	N ₀	0.55	0.75	0.24	0.21	0.47	0.09						
	N ₁₅₀	0.69	0.93	0.36	0.27	0.59	0.14						
	N ₂₀₀	0.72	1.00	0.38	0.28	0.63	0.15						
C ₇₅₀	N ₀	0.53	0.72	0.22	0.20	0.49	0.09						
	N ₁₅₀	0.71	0.91	0.35	0.28	0.63	0.14						
	N ₂₀₀	0.74	0.96	0.37	0.29	0.67	0.14						
Mean	C _{amb}	0.65	0.95	0.36	0.25	0.54	0.14						
	C ₅₅₀	0.65	0.89	0.33	0.25	0.56	0.13						
	C ₇₅₀	0.66	0.86	0.31	0.26	0.60	0.12						
	N ₀	0.55	0.75	0.24	0.22	0.47	0.09						
	N ₁₅₀	0.69	0.95	0.37	0.27	0.60	0.14						
	N ₂₀₀	0.72	1.01	0.39	0.28	0.63	0.15						
		SEd	CD	SEd	CD	SEd	CD						
C N C x N	C	0.005	NS	0.007	0.014**	0.003	0.006**	0.001	NS	0.003	0.007**	0.0008	0.001**
	N	0.005	0.010**	0.007	0.014**	0.003	0.006**	0.001	0.003**	0.003	0.007**	0.0008	0.001**
	C x N	0.009	0.019**	0.012	NS	0.005	NS	0.002	0.005**	0.006	NS	0.001	NS
CO ₂ levels : C _{amb} - 380 $\mu\text{mol mol}^{-1} \text{CO}_2$; C ₅₅₀ - 550 $\mu\text{mol mol}^{-1} \text{CO}_2$; C ₇₅₀ - 750 $\mu\text{mol mol}^{-1} \text{CO}_2$ Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹													

** $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

4.3.8.2. Glutamine Synthetase (GS)

The glutamine synthetase (GS) activity in leaves were significantly influenced by elevated CO₂ concentrations (Table 4.58). At tillering stage, the GS activity was slightly highest under elevated CO₂, but the values were non-significant. In the case of flowering and maturity stages, the GS activities in leaves were significantly highest in the plants exposed to elevated CO₂. The GS activities ranged from 1.81 to 2.48, 1.73 to 2.31 and 1.64 to 2.21 μ mol glutamyl hydroxamate min⁻¹ g⁻¹ f.wt in C_{amb}, C₅₅₀ and C₇₅₀ levels, respectively at flowering stage. The highest mean values in leaves (2.21 and 0.67 μ mol glutamyl hydroxamate min⁻¹ g⁻¹ f.wt) were recorded in C_{amb} while the lowest (1.98 and 0.59 μ mol glutamyl hydroxamate min⁻¹ g⁻¹ f.wt) in C₇₅₀ at flowering and maturity stages, respectively.

Nitrogen addition significantly increased the GS activities regardless of CO₂ levels or stages. The highest mean values (1.80, 2.33 and 0.74 μ mol glutamyl hydroxamate min⁻¹ g⁻¹ f.wt) were observed in N₂₀₀ and the lowest (1.38, 1.73 and 0.46 μ mol glutamyl hydroxamate min⁻¹ g⁻¹ f.wt) in control (N₀) at tillering, flowering and maturity stages, respectively. Among the stages, flowering recorded the highest GS activity in leaves and the lowest at maturity. The interaction effect was significant only at tillering stage.

The plants exposed to elevated CO₂ levels had significantly highest glutamine synthetase (GS) activity in roots at tillering and flowering stages while the trend was reversed at maturity. At maturity stage, the mean values were 0.77, 0.72 and 0.68 μ mol glutamyl hydroxamate min⁻¹ g⁻¹ f.wt in C_{amb}, C₅₅₀ and C₇₅₀, respectively. Nitrogen significantly increased the GS activity irrespective of CO₂ levels or stages. Among the stages, the highest GS activities in roots were observed at flowering and the lowest at harvest. The interaction effect was significant only at tillering stage.

4.3.8.3. Glutamate synthase (GOGAT)

The plants grown under elevated CO₂ concentrations had the highest glutamate synthase (GOGAT) activities in leaves at tillering stage but the values were non-significant (Table 4.59). On the other hand at flowering and maturity stages, the GOGAT activities in leaves were lowest under elevated CO₂ levels. The mean values were 2.62, 3.05, 1.05 μ mol glutamate min⁻¹ g⁻¹ f.wt in C₇₅₀ and 2.57, 3.37, 1.18 μ mol glutamate min⁻¹ g⁻¹ f.wt in C_{amb} at tillering, flowering and maturity stages, respectively.

Table 4.58. Effect of elevated CO₂ and nitrogen levels on Glutamine Synthetase (GS) activity in leaves and roots

Treatments		Leaves ($\mu\text{mol glutamyl hydroxamate min}^{-1} \text{ g}^{-1} \text{ f.wt}$)			Roots ($\mu\text{mol glutamyl hydroxamate min}^{-1} \text{ g}^{-1} \text{ f.wt}$)		
		Tillering	Flowering	Maturity	Tillering	Flowering	Maturity
C_{amb}	N₀	1.46	1.81	0.50	1.68	1.88	0.56
	N₁₅₀	1.63	2.33	0.73	1.89	2.39	0.84
	N₂₀₀	1.77	2.48	0.78	2.01	2.52	0.91
C₅₅₀	N₀	1.37	1.73	0.46	1.58	2.00	0.53
	N₁₅₀	1.72	2.16	0.69	1.97	2.49	0.79
	N₂₀₀	1.79	2.31	0.73	2.06	2.65	0.84
C₇₅₀	N₀	1.31	1.64	0.41	1.51	2.08	0.48
	N₁₅₀	1.78	2.10	0.66	2.05	2.62	0.76
	N₂₀₀	1.85	2.21	0.70	2.14	2.86	0.80
Mean	C_{amb}	1.62	2.21	0.67	1.86	2.26	0.77
	C₅₅₀	1.63	2.07	0.63	1.87	2.38	0.72
	C₇₅₀	1.65	1.98	0.59	1.90	2.52	0.68
	N₀	1.38	1.73	0.46	1.59	1.99	0.52
	N₁₅₀	1.71	2.20	0.69	1.97	2.50	0.80
	N₂₀₀	1.80	2.33	0.74	2.07	2.68	0.85
		SEd	CD	SEd	CD	SEd	CD
C	0.013	NS	0.017	0.005	0.010	0.014	0.004
N	0.013	0.027**	0.017	0.005	0.010	0.014	0.004
C x N	0.023	0.047**	0.029	0.009	0.019	0.024	0.007
CO ₂ levels : C _{amb} - 380 $\mu\text{mol mol}^{-1} \text{ CO}_2$; C ₅₅₀ - 550 $\mu\text{mol mol}^{-1} \text{ CO}_2$; C ₂ - 750 $\mu\text{mol mol}^{-1} \text{ CO}_2$							
Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹							
*P ≤ 0.05, **P ≤ 0.01, NS - Non significant							

Table 4.59. Effect of elevated CO₂ and nitrogen levels on Glutamate Synthase (GOGAT) activity in leaves and roots

Treatments		Leaves ($\mu\text{mol glutamate min}^{-1} \text{g}^{-1}$ fresh tissue)			Roots ($\mu\text{mol glutamate min}^{-1} \text{g}^{-1}$ fresh tissue)		
		Tillering	Flowering	Maturity	Tillering	Flowering	Maturity
C_{amb}	N₀	2.33	2.77	0.89	2.17	2.47	0.82
	N₁₅₀	2.62	3.56	1.27	2.46	3.05	1.17
	N₂₀₀	2.75	3.79	1.39	2.59	3.25	1.25
C₅₅₀	N₀	2.19	2.65	0.78	2.04	2.54	0.72
	N₁₅₀	2.66	3.30	1.21	2.50	3.15	1.12
	N₂₀₀	2.85	3.52	1.30	2.68	3.38	1.20
C₇₅₀	N₀	2.10	2.56	0.73	1.97	2.66	0.69
	N₁₅₀	2.84	3.20	1.16	2.67	3.42	1.07
	N₂₀₀	2.92	3.39	1.26	2.75	3.64	1.16
Mean	C_{amb}	2.57	3.37	1.18	2.41	2.92	1.08
	C₅₅₀	2.56	3.16	1.10	2.41	3.02	1.01
	C₇₅₀	2.62	3.05	1.05	2.46	3.24	0.97
	N₀	2.20	2.66	0.80	2.06	2.56	0.74
	N₁₅₀	2.71	3.36	1.22	2.54	3.21	1.12
	N₂₀₀	2.84	3.57	1.32	2.67	3.42	1.20
C N C x N	SEd	0.021	0.026	0.009	0.014	0.018	0.006
	CD	NS	0.053**	0.019**	0.029**	0.036**	0.012**
	SEd	0.021	0.026	0.009	0.014	0.018	0.006
C N C x N	CD	0.043**	0.053**	0.019**	0.029**	0.036**	0.012**
	SEd	0.037	0.045	0.016	0.024	0.031	0.010
	CD	0.075**	NS	NS	0.049**	0.063*	NS
CO ₂ levels : C _{amb} - 380 $\mu\text{mol mol}^{-1}$ CO ₂ ; C ₅₅₀ - 550 $\mu\text{mol mol}^{-1}$ CO ₂ ; C ₇₅₀ - 750 $\mu\text{mol mol}^{-1}$ CO ₂ Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹							

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

The plants exposed to elevated CO₂ levels did record significantly highest GOGAT activities in roots at tillering and flowering stages but the trend was reversed at maturity stage. Nitrogen addition significantly increased the GOGAT activities in leaves and roots irrespective of CO₂ levels or stages.

Among the stages, the highest GOGAT activities in leaves and roots were observed at flowering and the lowest at harvest. The interaction effect for leaves was significant only at tillering stage. With respect to the roots, the interaction effect was significant at tillering and flowering stages.

4.3.8.4. Glutamate Dehydrogenase (GDH)

The elevated CO₂ levels significantly decreased the glutamate dehydrogenase (GDH) activities in leaves regardless of stages of observation (Table 4.60). However the values were significant only at flowering and harvest stages. C_{amb} recorded the highest mean values (0.87, 1.26 and 0.47 μ mol NADH oxidized min⁻¹ g⁻¹ f.wt) while the lowest values (0.80, 1.14 and 0.41 μ mol NADH oxidized min⁻¹ g⁻¹ f.wt) in C₇₅₀ at tillering, flowering and harvest stages.

The plant exposed to elevated CO₂ levels had significantly highest GDH activities in roots at tillering stage. On the other hand at flowering and maturity stages, the GDH activities were similar at all levels of CO₂.

Nitrogen addition significantly increased the GDH activities in leaves irrespective of CO₂ levels or stages. With respect to roots, the nitrogen significantly increased GDH activities at tillering while there was no response at flowering and maturity stages.

Among the stages, the highest GDH activities in leaves and roots were observed at flowering and the lowest at harvest. The interaction effect was significant only at tillering stage in rice leaves. With respect to the roots, the interaction effect was non-significant.

4.3.9. Effect of elevated CO₂ levels on anti-oxidative enzyme activities

The plants grown under elevated CO₂ levels had recorded significantly lowest anti-oxidative enzyme activities viz., catalase, SOD (superoxide dismutase) and peroxidase at all stages of observation (Table 4.61). The highest mean catalase activities

Treatments		Leaves ($\mu\text{mol NADH oxidized min}^{-1} \text{ g}^{-1} \text{ f.wt}$)			Roots ($\mu\text{mol NADH oxidized min}^{-1} \text{ g}^{-1} \text{ f.wt}$)				
		Tillering	Flowering	Maturity	Tillering	Flowering	Maturity		
C _{amb}	N ₀	0.69	1.03	0.35	0.90	1.65	0.76		
	N ₁₅₀	0.94	1.33	0.51	1.27	1.67	0.74		
	N ₂₀₀	0.97	1.41	0.55	1.22	1.67	0.74		
C ₅₅₀	N ₀	0.66	0.99	0.32	0.86	1.63	0.75		
	N ₁₅₀	0.91	1.23	0.48	1.23	1.66	0.74		
	N ₂₀₀	0.95	1.31	0.51	1.18	1.64	0.73		
C ₇₅₀	N ₀	0.61	0.95	0.29	0.79	1.64	0.74		
	N ₁₅₀	0.87	1.20	0.46	1.21	1.63	0.73		
	N ₂₀₀	0.93	1.26	0.49	1.13	1.64	0.73		
Mean	C _{amb}	0.87	1.26	0.47	1.13	1.66	0.75		
	C ₅₅₀	0.84	1.18	0.44	1.09	1.64	0.74		
	C ₇₅₀	0.80	1.14	0.41	1.04	1.64	0.73		
	N ₀	0.65	0.99	0.32	0.85	1.64	0.75		
	N ₁₅₀	0.90	1.25	0.48	1.24	1.65	0.74		
	N ₂₀₀	0.95	1.33	0.51	1.18	1.65	0.73		
C N C x N		SEd	CD	SEd	CD	SEd	CD		
		0.007	NS	0.009	0.019**	0.006	0.013**	0.009	NS
		0.007	0.014**	0.009	0.019**	0.006	0.013**	0.009	NS
		0.012	0.025**	0.016	NS	0.006	NS	0.011	NS
CO ₂ levels : C _{amb} - 380 $\mu\text{mol mol}^{-1} \text{CO}_2$; C ₅₅₀ - 550 $\mu\text{mol mol}^{-1} \text{CO}_2$; C ₇₅₀ - 750 $\mu\text{mol mol}^{-1} \text{CO}_2$ Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹									

** $P \leq 0.05$, ** $P \leq 0.01$, NS - Non significant

Table 4.61. Effect of elevated CO₂ and nitrogen levels on anti-oxidative enzymes activities

Treatments	Catalase activity ($\mu\text{H}_2\text{O}_2\text{ g}^{-1}\text{ min}^{-1}$)			SOD activity (enzyme units $\text{mg}^{-1}\text{ protein min}^{-1}$)			Peroxidase activity ($\Delta 430\text{ nm g}^{-1}\text{ min}^{-1}$)		
	Tillering	Flowering	Harvest	Tillering	Flowering	Harvest	Tillering	Flowering	Harvest
C_{amb}	N₀	5.89	6.99	8.17	2.35	6.78	4.17	2.03	2.54
	N₁₅₀	5.25	5.98	7.11	2.10	5.80	3.63	1.81	2.21
	N₂₀₀	4.84	5.76	6.84	1.94	5.59	3.49	1.67	2.13
C₅₅₀	N₀	5.37	6.22	7.25	2.15	6.04	3.33	1.85	2.25
	N₁₅₀	4.93	5.83	7.10	1.97	5.65	3.26	1.70	2.21
	N₂₀₀	4.67	5.48	6.39	1.87	5.32	2.93	1.61	1.99
C₇₅₀	N₀	5.31	6.09	7.18	2.12	5.91	3.15	1.83	2.23
	N₁₅₀	4.76	5.24	6.31	1.90	5.08	2.77	1.64	1.96
	N₂₀₀	4.44	5.05	6.11	1.77	4.90	2.68	1.53	1.90
Mean	C_{amb}	5.33	6.25	7.38	2.13	6.06	3.76	1.84	2.29
	C₅₅₀	4.99	5.84	6.92	2.00	5.67	3.17	1.72	2.15
	C₇₅₀	4.83	5.46	6.53	1.93	5.29	2.87	1.67	2.03
	N₀	5.52	6.44	7.54	2.21	6.24	3.55	1.90	2.34
	N₁₅₀	4.98	5.68	6.84	1.99	5.51	3.22	1.72	2.13
	N₂₀₀	4.65	5.43	6.45	1.86	5.27	3.03	1.60	2.00
C x N	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd
	0.014	0.029**	0.017	0.034**	0.017	0.034**	0.027	0.042	0.057
	0.014	0.029**	0.017	0.034**	0.017	0.034**	0.027	0.042	0.057
C x N	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd
	0.025	NS	0.029	0.059*	0.031	0.063*	0.046	0.072	0.099
	0.025	NS	0.029	0.059*	0.031	0.063*	0.046	0.072	0.099

CO₂ levels : C_{amb} - 380 $\mu\text{mol mol}^{-1}\text{ CO}_2$; C₅₅₀ - 550 $\mu\text{mol mol}^{-1}\text{ CO}_2$; C₇₅₀ - 750 $\mu\text{mol mol}^{-1}\text{ CO}_2$ Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

(5.33, 6.25 and 7.38 $\mu\text{H}_2\text{O}_2\text{ g}^{-1}\text{min}^{-1}$) were observed in C_{amb} and the lowest values (4.83, 5.46 and 6.53 $\mu\text{H}_2\text{O}_2\text{ g}^{-1}\text{min}^{-1}$) in C_{750} at tillering, flowering and harvest stages, respectively. A similar trend of response was observed for SOD and peroxidase activities.

Incremental doses of nitrogen addition significantly reduced the anti-oxidative enzyme activities regardless of stages. N_0 registered the highest anti-oxidative enzyme activities while the lowest in N_{200} . The control (N_0) plants recorded the highest mean SOD activities (2.21, 6.24 and 3.55 enzyme units $\text{mg}^{-1}\text{protein min}^{-1}$) and the lowest values (1.86, 5.27 and 3.03 enzyme units $\text{mg}^{-1}\text{protein min}^{-1}$) in N_{200} . Similar trend of responses were observed for catalase and peroxidase activities as a result of nitrogen addition. The interaction effect was significant at flowering and harvest stages for all the three anti-oxidative enzyme activities.

4.3.10. Effect of elevated CO_2 levels on yield attributes

4.3.10.1. Productive tillers hill^{-1}

Rice plants exposed to elevated CO_2 levels recorded significantly highest number of productive tillers hill^{-1} (Table 4.62). C_{750} registered the highest number of productive tillers hill^{-1} ranging from 7.2 to 11.2 at harvest, followed by C_{550} (6.8 to 10.2) and the lowest (6.0 to 8.4) in C_{amb} . The mean values are 9.6, 8.9 and 7.4 in C_{750} , C_{550} and C_{amb} , respectively.

Application of nitrogen significantly increased the highest number of productive tillers hill^{-1} . The highest mean value (9.9) was observed in N_{200} followed by N_{150} (9.4) and the lowest (6.7) in control (N_0). The interaction effect was significant.

4.3.10.2. Days to 50 per cent flowering

The observations revealed that the plants exposed to elevated CO_2 levels had early flowering compared to plants grown under ambient CO_2 concentration (Table 4.62). In C_{550} , the mean number of days to 50 per cent flowering was 80.1, followed by C_{750} (80.2) and the last one in the order was C_{amb} (82.2). The days to 50 per cent flowering was not significantly influenced as a result of nitrogen addition. The interaction effect was non-significant.

Table 4.62. Effect of elevated CO₂ and nitrogen levels on yield attributes

Treatments		Productive tillers per hill	Days to 50% flowering	Grains (number panicle ⁻¹)	Filled grains (number panicle ⁻¹)	1000 grain weight (g)	
C _{amb}	N ₀	6.0	82.1	95	70	17.6	
	N ₁₅₀	7.9	82.0	109	84	17.6	
	N ₂₀₀	8.4	82.4	116	91	17.7	
C ₅₅₀	N ₀	6.8	80.2	97	78	17.6	
	N ₁₅₀	9.7	80.0	123	103	17.7	
	N ₂₀₀	10.2	80.1	130	112	17.7	
C ₇₅₀	N ₀	7.2	80.3	101	81	17.6	
	N ₁₅₀	10.5	80.1	131	114	17.7	
	N ₂₀₀	11.2	80.2	139	123	17.7	
Mean	C _{amb}	7.4	82.2	107	82	17.6	
	C ₅₅₀	8.9	80.1	117	98	17.6	
	C ₇₅₀	9.6	80.2	123	106	17.7	
	N ₀	6.7	80.9	98	76	17.6	
	N ₁₅₀	9.4	80.7	121	100	17.7	
	N ₂₀₀	9.9	80.9	128	109	17.7	
		SEd	CD	SEd	CD	SEd	CD
C	0.07	0.15**	0.47	0.07	0.06	0.14	NS
N	0.07	0.15**	0.47	0.07	0.06	0.14	NS
C x N	0.12	0.25**	0.81	0.14	0.10	0.25	NS

CO₂ levels : C_{amb} - 380 μ mol mol⁻¹ CO₂; C₅₅₀ - 550 μ mol mol⁻¹ CO₂; C₇₅₀ - 750 μ mol mol⁻¹ CO₂
Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹

CO₂ levels : C_{amb} - 380 μmol mol⁻¹ CO₂; C₅₅₀ - 550 μmol mol⁻¹ CO₂; C₂ - 750 μmol mol⁻¹ CO₂
 Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹

*P ≤ 0.05, **P ≤ 0.01, NS - Non significant

4.3.10.3. Grains

Rice plants exposed to $750 \mu \text{mol mol}^{-1} \text{CO}_2$ had significantly recorded the highest number of grains panicle⁻¹ (101 to 139), followed by $550 \mu \text{mol mol}^{-1} \text{CO}_2$ (97 to 130) and the lowest (95 to 116) in $380 \mu \text{mol mol}^{-1} \text{CO}_2$ (Table 4.62). Incremental levels of nitrogen significantly increased the number of grains panicle⁻¹. N_{200} recorded the highest mean value (128), while the lowest (98) in control (N_0). The interaction effect was significant.

4.3.10.4. Filled grains

The number of filled grains panicle⁻¹ was significantly highest under C_{750} followed by C_{550} and the lowest in C_{amb} (Table 4.62). The mean values are 82, 98 and 106 in C_{amb} , C_{550} and C_{750} , respectively. The application of nitrogen significantly increased the number of filled grains panicle⁻¹. The highest number of filled grains panicle⁻¹ (91 to 123) was recorded in N_{200} and the lowest (70 to 81) in control (N_0). The interaction effect was significant.

4.3.10.5. Thousand grain weight

The thousand grain weight of rice grains was unaltered regardless of CO_2 concentrations or nitrogen doses. The values were non-significant (Table 4.62).

4.3.10.6. Yield

Plants exposed to elevated levels of CO_2 had recorded significantly highest grain, straw and root yields in comparison to plants under ambient concentration (Table 4.63). The grain yield ranged from 20.7 to 34.7, 21.7 to 44.7 and 23.1 to 52.0 g pot⁻¹ in C_{amb} , C_{550} and C_{750} levels, respectively. C_{750} increased the grain, straw and root yields by 40.1 and 12.9 per cent, 15.6 and 5.7 per cent, 54.8 and 15.3 per cent, over ambient (C_{amb}) and C_{550} , respectively. The grain, straw and root yields were increased in C_{550} by 24.0, 9.3, 34.2 per cent over ambient (C_{amb}).

Root : shoot ratio was significantly increased in the plants exposed to elevated CO_2 concentrations. Plant under $750 \mu \text{mol mol}^{-1} \text{CO}_2$ increased the root:shoot ratio by 30.9 and 7.9 per cent over ambient ($380 \mu \text{mol mol}^{-1} \text{CO}_2$) and $550 \mu \text{mol mol}^{-1} \text{CO}_2$, respectively.

Nitrogen significantly increased the grain, straw and root yields irrespective of CO_2 concentrations. The highest mean grain, straw and root yields (43.8, 69.8 and 11.3 g pot⁻¹)

Table 4.63. Effect of elevated CO₂ and nitrogen levels on yield

Treatments		Grain yield (g pot ⁻¹)		Straw yield (g pot ⁻¹)		Root yield (g pot ⁻¹)		Root : Shoot ratio	
C _{amb}	N ₀	20.7		40.5		5.9		0.146	
	N ₁₅₀	30.8		59.1		7.7		0.130	
	N ₂₀₀	34.7		64.3		8.4		0.131	
C ₅₅₀	N ₀	21.7		43.5		7.3		0.168	
	N ₁₅₀	40.4		65.4		10.5		0.161	
	N ₂₀₀	44.7		70.1		11.6		0.165	
C ₇₅₀	N ₀	23.1		45.2		7.7		0.170	
	N ₁₅₀	45.5		69.2		12.4		0.179	
	N ₂₀₀	52.0		75.0		13.8		0.184	
Mean	C _{amb}	28.7		54.6		7.3		0.136	
	C ₅₅₀	35.6		59.7		9.8		0.165	
	C ₇₅₀	40.2		63.1		11.3		0.178	
	N ₀	21.8		43.1		7.0		0.161	
	N ₁₅₀	38.9		64.6		10.2		0.157	
	N ₂₀₀	43.8		69.8		11.3		0.160	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD
C		0.21	0.43**	0.35	0.71**	0.63	1.28**	0.001	0.002**
N		0.21	0.43**	0.35	0.71**	0.63	1.28**	0.001	0.002*
C x N		0.36	0.74**	0.60	1.22**	1.09	NS	0.002	0.003**
CO ₂ levels : C _{amb} - 380 μ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μ mol mol ⁻¹ CO ₂ ; C ₇₅₀ - 750 μ mol mol ⁻¹ CO ₂									
Nitrogen levels : N ₀ – 0 kg N ha ⁻¹ ; N ₁₅₀ – 150 kg N ha ⁻¹ ; N ₂₀₀ – 200 kg N ha ⁻¹									

*P ≤ 0.05, ** P ≤ 0.01, NS - Non significant

were registered in N₂₀₀, followed by N₁₅₀ (38.9, 64.6 and 10.2 g pot⁻¹) and the lowest values (21.8, 43.1 and 7.0 g pot⁻¹) in control (N₀). The root:shoot ratio was highest in N₂₀₀ and the lowest in control (N₀). The interaction effect was significant.

4.3.11. Effect of elevated CO₂ levels on plant carbon and nitrogen

4.3.11.1. Carbon content

The carbon content in roots and straw were significantly increased in the plants exposed to elevated CO₂ concentrations (Table 4.64). However, the carbon content in grains was unaltered. The highest mean carbon contents (41.9 and 41.0 per cent) were recorded in C₇₅₀ while the lowest values (38.6 and 35.2 per cent) in roots and straw, respectively. In general, the carbon content in grains ranged between 42.7 and 43.2 per cent, but the values were non-significant. The interaction effect was absent.

4.3.11.2. Nitrogen content

The plants exposed to elevated CO₂ levels registered significantly lowest nitrogen content in roots, straw and grains at maturity stage (Table 4.64). The highest mean nitrogen values (0.58, 0.89 and 1.35 per cent) were observed in C_{amb} and the lowest values (0.51, 0.80 and 1.27 per cent) in C₇₅₀ in roots, straw and grains, respectively. Nitrogen addition significantly increased the nitrogen contents. The highest mean nitrogen values (0.59, 0.92 and 1.43 per cent) were registered in N₂₀₀, followed by N₁₅₀ (0.55, 0.90 and 1.35 per cent) and the lowest values (0.71, 0.48 and 1.13 per cent) in control (N₀) in roots, straw and grains. The interaction effect was significant.

4.3.11.3. Biomass carbon

Plants exposed to elevated levels of CO₂ had recorded significantly highest biomass carbon in roots, straw and grains (Table 4.65). C₇₅₀ registered highest mean biomass carbon (4.48, 25.91 and 17.20 g pot⁻¹) followed by C₅₅₀ (3.79, 23.55 and 15.31 g pot⁻¹) and the lowest values (3.07, 19.22 and 12.30 g pot⁻¹) in C_{amb} were registered in roots, straw and grains, respectively. The addition of nitrogen significantly increased the plant biomass carbon. The highest mean biomass carbon (4.49, 27.02 and 18.84 g pot⁻¹) in N₂₀₀ while the lowest values (2.78, 16.64 and 9.34 g pot⁻¹) were registered in control (N₀). The interaction effect was significant.

Treatments		Carbon content (%)			Nitrogen content (%)						
		Straw	Roots	Grains	Straw	Roots	Grains				
C _{amb}	N ₀	35.2	38.6	42.7	0.79	0.53	1.26				
	N ₁₅₀	35.1	38.6	42.9	0.93	0.60	1.38				
	N ₂₀₀	35.2	38.7	42.9	0.96	0.61	1.42				
C ₅₅₀	N ₀	39.4	39.5	42.8	0.70	0.48	1.09				
	N ₁₅₀	39.5	39.7	42.9	0.90	0.54	1.32				
	N ₂₀₀	39.5	39.7	43.2	0.92	0.59	1.44				
C ₇₅₀	N ₀	41.0	41.9	42.9	0.63	0.44	1.03				
	N ₁₅₀	41.1	41.9	42.7	0.87	0.52	1.36				
	N ₂₀₀	41.0	41.8	42.9	0.89	0.56	1.43				
Mean	C _{amb}	35.2	38.6	42.8	0.89	0.58	1.35				
	C ₅₅₀	39.5	39.6	43.0	0.84	0.54	1.28				
	C ₇₅₀	41.0	41.9	42.8	0.80	0.51	1.27				
	N ₀	38.5	40.0	42.8	0.71	0.48	1.13				
	N ₁₅₀	38.6	40.1	42.8	0.90	0.55	1.35				
	N ₂₀₀	38.6	40.1	43.0	0.92	0.59	1.43				
		SEd	CD	SEd	CD	SEd	CD				
C	0.22	0.45**	0.23	0.47**	0.005	0.009**	0.003	0.006**	0.007	0.015**	
N	0.22	NS	0.23	NS	0.005	0.009**	0.003	0.006**	0.007	0.015**	
C x N	0.38	NS	0.40	NS	0.008	0.017**	0.005	0.011*	0.013	0.027**	
CO ₂ levels : C _{amb} - 380 μ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 μ mol mol ⁻¹ CO ₂ ; C ₂ - 750 μ mol mol ⁻¹ CO ₂ Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹											

CO₂ levels : C_{amb} - 380 μmol mol⁻¹ CO₂; C₅₅₀ - 550 μmol mol⁻¹ CO₂; C₂₋₇₅₀ μmol mol⁻¹ CO₂
 Nitrogen levels : N₀ - 0 kg N ha⁻¹; N₁₅₀ - 150 kg N ha⁻¹; N₂₀₀ - 200 kg N ha⁻¹
 *P ≤ 0.05, **P ≤ 0.01, NS - Non significant

Table 4.65. Effect of elevated CO₂ and nitrogen levels on biomass carbon and nitrogen uptake

Treatments		Biomass carbon (g pot ⁻¹)			Nitrogen uptake (g pot ⁻¹)					
		Shoot	Root	Grain	Shoot	Root	Grain			
C _{amb}	N ₀	14.26	2.47	8.82	0.32	0.03	0.26			
	N ₁₅₀	20.76	3.23	13.19	0.55	0.05	0.42			
	N ₂₀₀	22.62	3.51	14.89	0.62	0.05	0.49			
	N ₀	17.13	2.82	9.31	0.30	0.04	0.24			
C ₅₅₀	N ₁₅₀	25.83	4.05	17.31	0.59	0.06	0.53			
	N ₂₀₀	27.69	4.49	19.32	0.64	0.07	0.64			
	N ₀	18.54	3.04	9.89	0.28	0.03	0.24			
C ₇₅₀	N ₁₅₀	28.44	4.92	19.42	0.60	0.06	0.62			
	N ₂₀₀	30.75	5.48	22.30	0.67	0.08	0.74			
Mean	C _{amb}	19.22	3.07	12.30	0.50	0.04	0.39			
	C ₅₅₀	23.55	3.79	15.31	0.51	0.05	0.47			
	C ₇₅₀	25.91	4.48	17.20	0.52	0.06	0.53			
	N ₀	16.64	2.78	9.34	0.30	0.03	0.24			
	N ₁₅₀	25.01	4.07	16.64	0.58	0.06	0.53			
	N ₂₀₀	27.02	4.49	18.84	0.64	0.07	0.63			
C N C x N	SEd	CD	SEd	CD	SEd	CD	SEd	CD		
	0.136	0.275**	0.022	0.046**	0.090	0.183**	0.003	0.0007**	0.003	0.006**
	0.136	0.275**	0.022	0.046**	0.090	0.183**	0.003	0.0007**	0.003	0.006**
	0.235	0.477**	0.039	0.079**	0.156	0.317**	0.005	0.010**	0.006	0.010**
CO ₂ levels : C _{amb} - 380 µ mol mol ⁻¹ CO ₂ ; C ₅₅₀ - 550 µ mol mol ⁻¹ CO ₂ ; C ₇₅₀ - 750 µ mol mol ⁻¹ CO ₂ Nitrogen levels : N ₀ - 0 kg N ha ⁻¹ ; N ₁₅₀ - 150 kg N ha ⁻¹ ; N ₂₀₀ - 200 kg N ha ⁻¹ *P ≤ 0.05; ** P ≤ 0.01; NS - Non significant										

4.3.11.4. Nitrogen uptake

The nitrogen uptake was significantly increased in plants grown under elevated levels of CO₂ (Table 4.65). The highest mean nitrogen uptake (0.06, 0.52 and 0.53 g pot⁻¹) were observed in C₇₅₀ while the lowest values (0.04, 0.50 and 0.39 g pot⁻¹) in C_{amb} were recorded in roots, straw and grains, respectively. Incremental levels of nitrogen addition significantly increased the nitrogen uptake. The highest mean nitrogen values (0.07, 0.64 and 0.63 g pot⁻¹) were registered in N₂₀₀, followed by N₁₅₀ (0.06, 0.58 and 0.53 g pot⁻¹) and the lowest values (0.03, 0.30 and 0.24 g pot⁻¹) in control (N₀). The interaction effect was significant.

Discussion

CHAPTER 5

DISCUSSION

The carbon sequestration pattern and methane emission of two distinct systems of rice cultivation namely SRI and conventional were assessed through two field experiments (summer and *kharif*, 2011) and a laboratory study at the Department of Environmental Sciences, TNAU, Coimbatore. In addition to these experiments, open top chamber (OTC) study was taken up in order to determine the response of rice plants to elevated CO₂ levels under varying levels of nitrogen. The data collected from these experiments were statistically analyzed and the results obtained are critically discussed in this section.

5.1. Carbon sequestration pattern in rice soils

The carbon dynamics in the soils of conventional and SRI systems of rice cultivation were assessed in terms of active and passive pools of carbon and carbon stock accumulation. This study critically evaluated the carbon distribution pattern in two distinct systems of rice cultivation under varying levels of nitrogen. The temporal and seasonal changes in carbon flow in rice soils were monitored in order to gain insights into the carbon sequestration pattern. The data on active carbon pools (microbial biomass carbon and dissolved organic carbon) and passive pools of carbon (humic acid, fulvic acid, humin and glomalin) besides soil organic carbon in the furrow slice of rice soils were quantified.

5.1.1. Active carbon pools

The data on active carbon pools especially the soil microbial biomass carbon (SMBC) indicated that SRI system of rice cultivation had consistently higher values in comparison to conventional system irrespective of the stages of observation (Fig.5.1a and 5.1b). It is quite understandable that SRI system possesses favourable environment and crop anatomical features that facilitate retention of higher levels of SMBC. Since SRI encompasses cono-weeding practice which churns the soil repeatedly at least four times during the cropping season that aerates the soil and assists in stimulated microbial activities. These processes would have helped SRI soils to retain higher levels of SMBC. Our data are in agreement with the observations of Uphoff *et al.* (2009) and Iswandi *et al.* (2011) who have reported close relationship between cono-weeding and intense biological

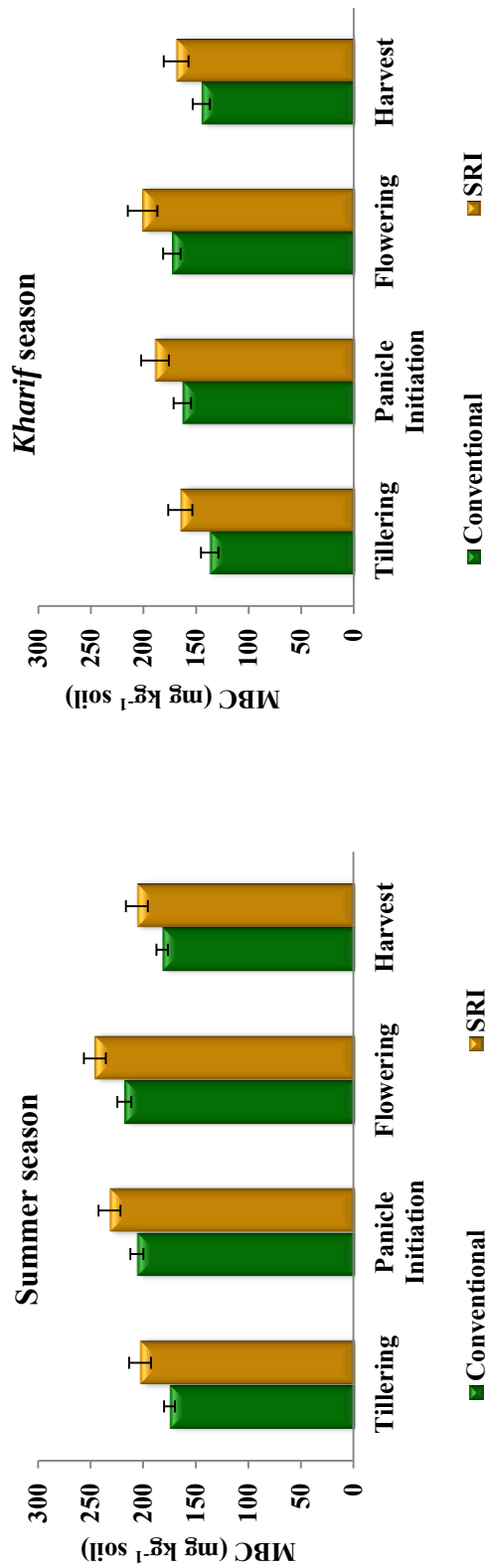


Fig.5.1a. Effect of systems of rice cultivation on microbial biomass carbon at rice growth stages

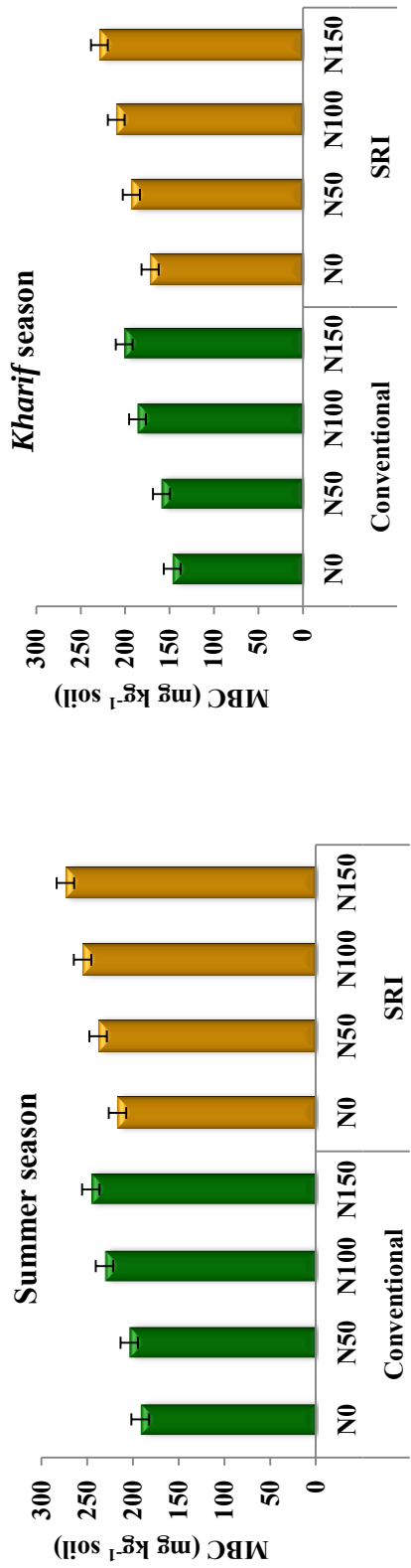


Fig.5.1b. Effect of systems of rice cultivation and nitrogen levels on microbial biomass carbon at flowering stages

activities *vis-à-vis* SMBC. Further, SRI system is exposed to intermittent wet and dry regime of moisture that also triggers the microbial activities which in turn contribute for the enhanced SMBC. The results of our study closely coincided with the observations of Yang *et al.* (2005) and Zhao *et al.* (2010) who have suggested alternate wet and dry regime accentuated microbial activities and SMBC. In addition to these changes in soils, the morphological features of SRI rice had highly branched fibrous root system that harbor's intense microbial activity in the rhizosphere. Our data have shown that SRI system of rice cultivation increased the root biomass per hill by 76 to 100 per cent over the conventional system. These favourable changes helped SRI to maintain higher SMBC throughout the cropping season. On the other hand, the dissolved organic carbon (DOC) measured was lower in SRI system (Fig.5.2a and 5.2b). This may be attributed to the exploitation of high energy carbon source by the microbes in SRI. Moreover, the DOC concentration in soil solution may be controlled by the interactive effect of biotic and abiotic factors (Kalbitz *et al.*, 2000; Luo *et al.*, 2009) and hence it is usually reported as the major sources of labile C in flooded soils (Lu *et al.*, 2004; Zschornack *et al.*, 2011).

Conversely, in conventional system of rice cultivation, the soil is kept under submergence during major part of the cropping season that restricts the biological activity of the soil. Since, the soil is exposed to submergence, only a set of anaerobes could survive under these conditions. This statement is strongly supported by our measurements on total anaerobes population which is 6.5 to 30.5 per cent higher than SRI system of rice cultivation. Our data are in agreement with some of the classical literature reported by Ponnamperna (1972) who indicated lower redox potential values for submerged soils.

The active carbon pools in SRI and conventional systems were evaluated in two distinct seasons and different levels of nitrogen. The incremental levels of N fertilization increased both SMBC and DOC throughout the cropping season irrespective of the rice cultivation system. It is obvious that addition of incremental levels of nitrogen favoured microbial activities that would have eventually resulted in higher SMBC. It is well established that there is a close relationship between SMBC and nitrogen (Rupela *et al.*, 2006; Zhao *et al.*, 2010). The higher DOC as a result of the nitrogen addition is due to the

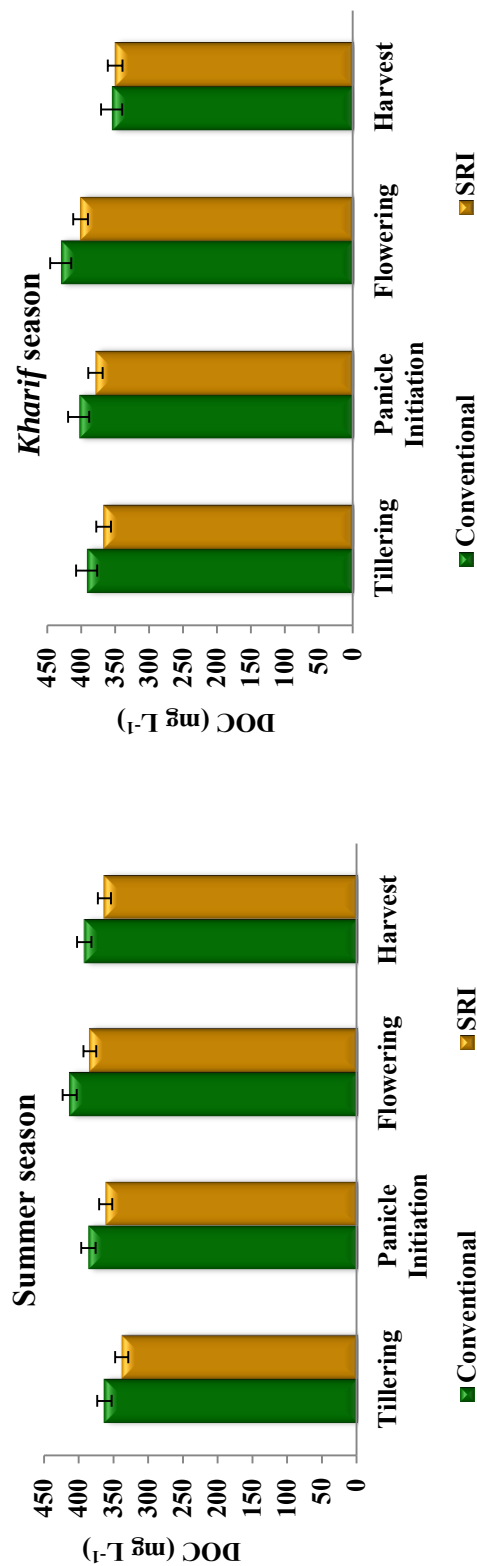


Fig.5.2a. Effect of systems of rice cultivation on dissolved organic carbon at rice growth stages

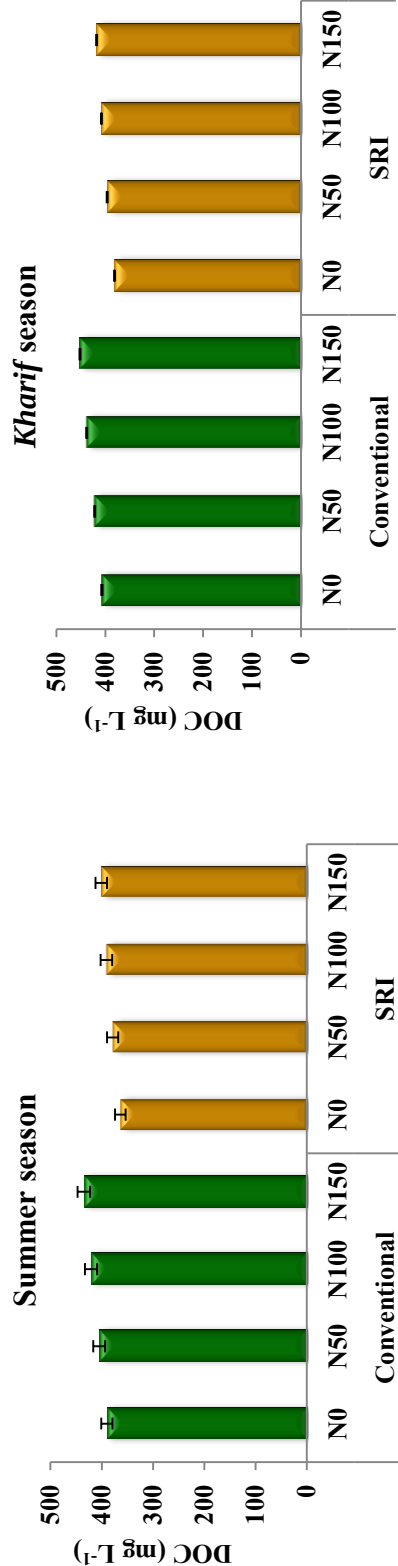


Fig.5.2b. Effect of systems of rice cultivation and nitrogen levels on DOC at flowering stages

fact that nitrogen favoured better enhanced rice biomass and root exudates, which in turn contributed for higher DOC contents. This is in line with Lu *et al.* (2004) who estimated that more than 34 per cent of DOC came from photosynthesis during rice growth.

5.1.2. Passive carbon pools

The passive carbon pool encompasses humic acid, fulvic acid, humin and glomalin that are recalcitrant and directly contribute for the permanent pool of soil organic carbon. Our study has investigated these parameters during the course of cropping season in two distinct systems of rice cultivation. The data suggested that SRI has unique advantage of retaining higher levels of passive pools of carbon in comparison to conventional system of rice cultivation (Fig.5.3). The SRI system has unique features such as wetting and drying and cono-weeding practice that are either favourable to enhanced humification processes or rapid decomposition of non-humic substances which might have contributed for higher humic acid and fulvic contents of SRI than conventional system of rice cultivation.

Soil organic matter has two major types of compounds: (1) non-humic substances, belonging to identifiable chemical classes such as carbohydrates, and (2) humic substances consisting of a series of brown to dark-brown, high-molecular weight bio-polymers (Quideau, 2002). The conventional definition of humification is a gradual reworking of the organic matter through oxidative and hydrolytic reactions mediated by soil microorganisms and possibly also through various abiotic reactions such that labile compounds are depleted over time in favour of more recalcitrant compounds (Stevenson, 1994; Zech *et al.*, 1997). The formation of humic compounds is maximized under partly oxidizing conditions: If there is too much oxygen, full mineralization occurs; if there is too little, oxidative polymerization is stifled. Frequent wetting and drying cycles avoid the stagnation that occurs under either oxidizing or reducing conditions and promote the oxidative polymerization reaction that stabilizes carbon (Post *et al.*, 2004). These facts strengthen out statement that SRI soils with relatively aerobic conditions as a result of alternate wetting and drying irrigation management might favoured for improving the passive pools of carbon through enhanced humification processes. In addition, it is also attributed that the aerobic nature of SRI soils along with cono-weeding operation might facilitate

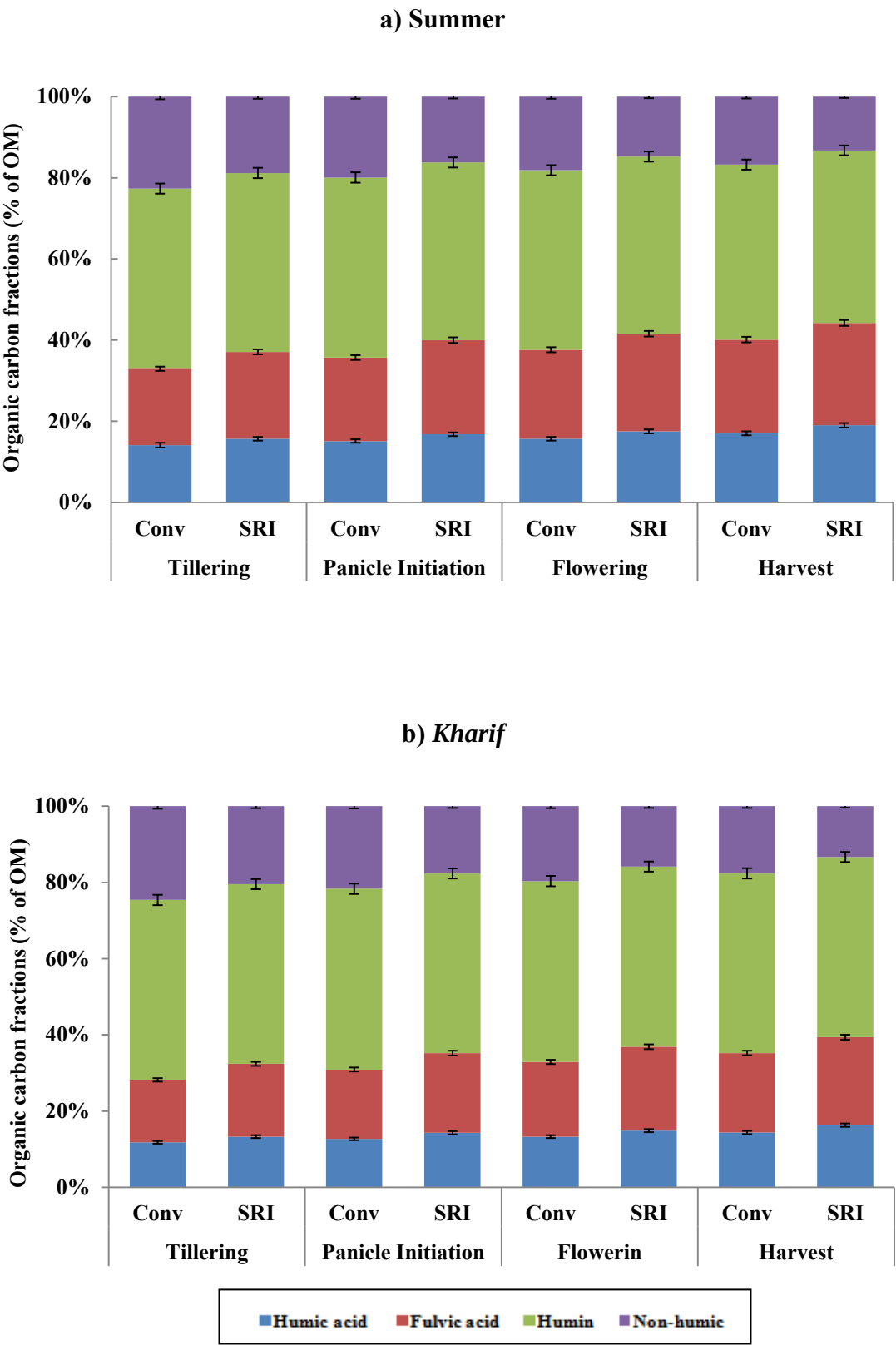


Fig. 5.3. Effect of systems of rice cultivation on soil organic carbon fractions

for rapid decomposition of non-humic substances. This is in agreement with Koivula and Hanninen (2001) who reported that the non-humic substances like carbohydrates are rapidly reduced during humification process.

It is further explained that the components of SRI system such as alternate wetting and drying water management that provides irrigation after the development of hair line crack and the practice of cono-weeding that churns and aerates the soil are the key factors that facilitate humification process through the oxidation of phenolic substances in rice soils. This is in accordance with the findings of Stevenson (1994) who reported that the sequential two-step nature of the humification process is the oxidation of phenolic groups followed by condensation of the resulting quinones with amino acids to form melanins. Importantly, the rate-limiting step in the humification process appears to be the oxidation of polyphenols to quinones. These quinones then react with peptides and amino acids to form large melanin-like polymers that resist further degradation by microorganisms. In addition, the high pH enhances the humification process primarily through its effect on the condensation step (Amonette *et al.*, 2004). This literature is in conformity with the observations of the present study where the experimental soils had alkaline pH that would have favoured condensation step of the humification process. Similar trend of response was also seen for fulvic acid.

The passive pools of carbon in conventional system of rice cultivation have shown to be lower than SRI throughout the course of investigation. The lower levels of passive pools of carbon in conventional system vividly explain the suppression of humification which is favoured in the other system of rice cultivation. It is well known that continuous submergence restricts oxidation or decomposition of organic matter, biological activity besides excluding major portion of microbes that catalyze humification process. Our data support that submergence inhibits humification and this might have resulted in lowering of passive pools of carbon in conventional system. Our findings closely coincide with the observations of reported literatures (Ponnamperuma, 1972; Sahrawat, 2004b). It is further supported by Maie *et al.* (2000) who reported that the organic matter became less humified with the increasing intensity of irrigated rice cropping and soil submergence. With increasing submergence, the humic acid fractions became less polycondensed and less oxidized or humified with higher sulfur and

hydrogen and lower oxygen concentrations. Mahieu *et al.* (2002) also demonstrated that regular aeration of soils promoted soil organic matter humification and that the humic acid fractions were less humified with increasing intensity of irrigated rice cropping. In contrast, the humin content of soils of conventional system was maintained higher than SRI that can be explained as a resultant product of long term decomposition process. Since the submergence was continuous for a period of more than three months a portion of decomposition should have been completed and eventually contributed for humin fraction while such may not have taken place in SRI where passive pools have been transiently increased as a consequence of carbon transformation.

In addition to humic acid and fulvic acid fractions, glomalin was also measured in our study. Glomalin is a glyco-protein and a super glue which encourages soil aggregate stability and thereby retaining carbon in the soil for an extended period of time. Despite the fact that glomalin is derived from plant mycorrhizal symbiosis (Subramanian *et al.*, 2009), the SRI system favoured the colonization of indigenous mycorrhizal colonization that might have helped the SRI system to maintain higher levels of glomalin in comparison to conventional system. Passive soil aeration, resulting from not keeping fields continuously flooded and letting them dry out, even to the surface-cracking point, several times during plants vegetative growth, is surely necessary for active soil aeration to be effective. When soil is mostly aerobic, mycorrhizal fungi can thrive and provide rice crops with the benefits that most other terrestrial plants receive from these symbionts' services. Further, it is reported that even in unfavorable flooded soil environments, mycorrhizal inoculation of irrigated rice plants can increase their yield by 10% (Solaiman and Hirata, 1997). Presumably even more benefit can be obtained from enhancing mycorrhizal fungi in rice roots under SRI which have more aerobic soil conditions (Uphoff *et al.*, 2009). Mycorrhizal associations may also be contributing to plant nutrition with SRI practices as these symbiotic fungi, which require aerobic soil conditions, can greatly increases the volume of soil from which plant roots can acquire nutrients (Sieverding, 1991; Pinton *et al.*, 2000).

The nitrogen fertilization had favoured passive pools of soil organic carbon regardless of systems of rice cultivation. Nitrogen being the source of mineral nutrition of microbes, the incremental levels of nitrogen would have linearly increased the microbial

activities that are involved in decomposition/humification process (Kuznetsova *et al.*, 1998; Fisk and Fahey, 2001).

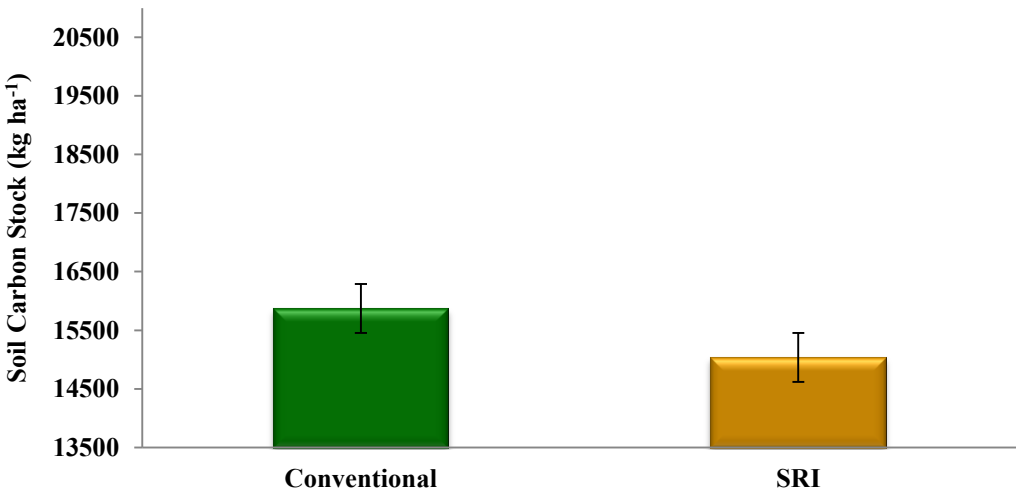
5.1.3. Soil carbon stock

With a view to assess the carbon sequestration potential of two distinct system of rice cultivation, SOC was measured periodically. The data have shown that carbon stock in conventional system of rice cultivation had higher values than SRI (Fig.5.4). Despite the fact that SRI had maintained consistently higher values for both active and passive pools of carbon with an exception of DOC and humin, the soils in conventional system had higher SOC. The rate of soil organic matter decomposition is lessened in lowland rice fields, apparently due to excessively reduced conditions (Watanabe, 1984). Because of the lack of oxygen under submerged conditions even a modest oxygen demand for microbial activity cannot be met if large pores are filled with water, resulting in a decreased rate of decomposition (Jenkinson, 1988). Therefore, there is incomplete decomposition of organic materials and decreased humification of organic matter under submerged conditions, resulting in net accumulation of organic matter in soils (Sahrawat, 2004b). Bronson *et al.* (1998) indicated a possible conservation of or increase in C stocks in soil in the lowland tropics due to the relatively slow rate of soil C mineralization under anaerobic conditions.

Carbon accumulation in continuous rice cropping may be favored by the accumulation of phenolic end products (Olk *et al.*, 1996). Oxidation of phenolic subunits produced from lignin degradation is greatly retarded when oxygen is limiting (Colberg, 1988), allowing phenols to accumulate in intensively cropped rice soils. In aerated soils, by contrast, phenolic compounds can be completely degraded. Consistent with this hypothesis, Olk *et al.* (1996) found greater phenol-C content in young humic acid fractions extracted from soils with a history of continuous irrigated rice cropping.

In order to support that relationship between submergence and SOC accumulation, we conducted incubation study with two levels of moisture regimes simulating the field conditions. Our data have corroborated with the information that SOC retained higher when the soil was kept under continuous submergence in comparison to alternate wetting and drying conditions. These data strongly support that submergence assisted in accumulation of SOC under field conditions.

a) Summer



b) Kharif

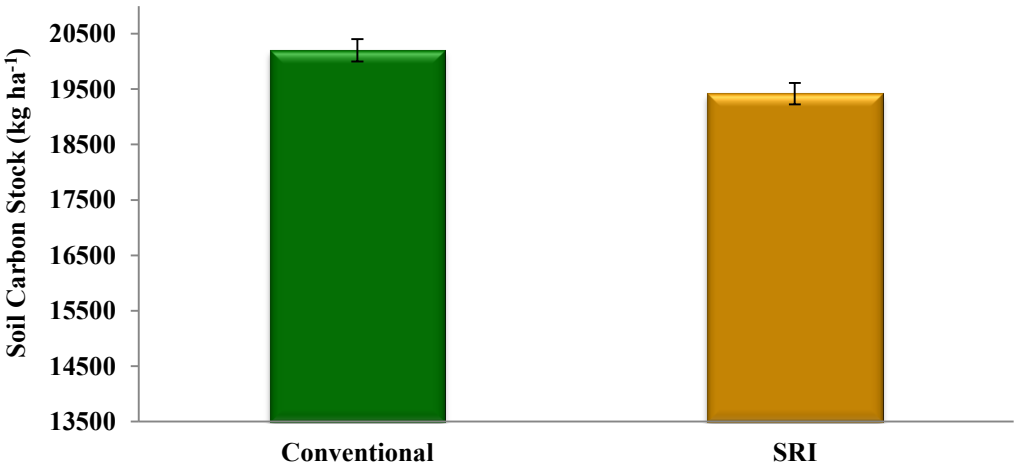
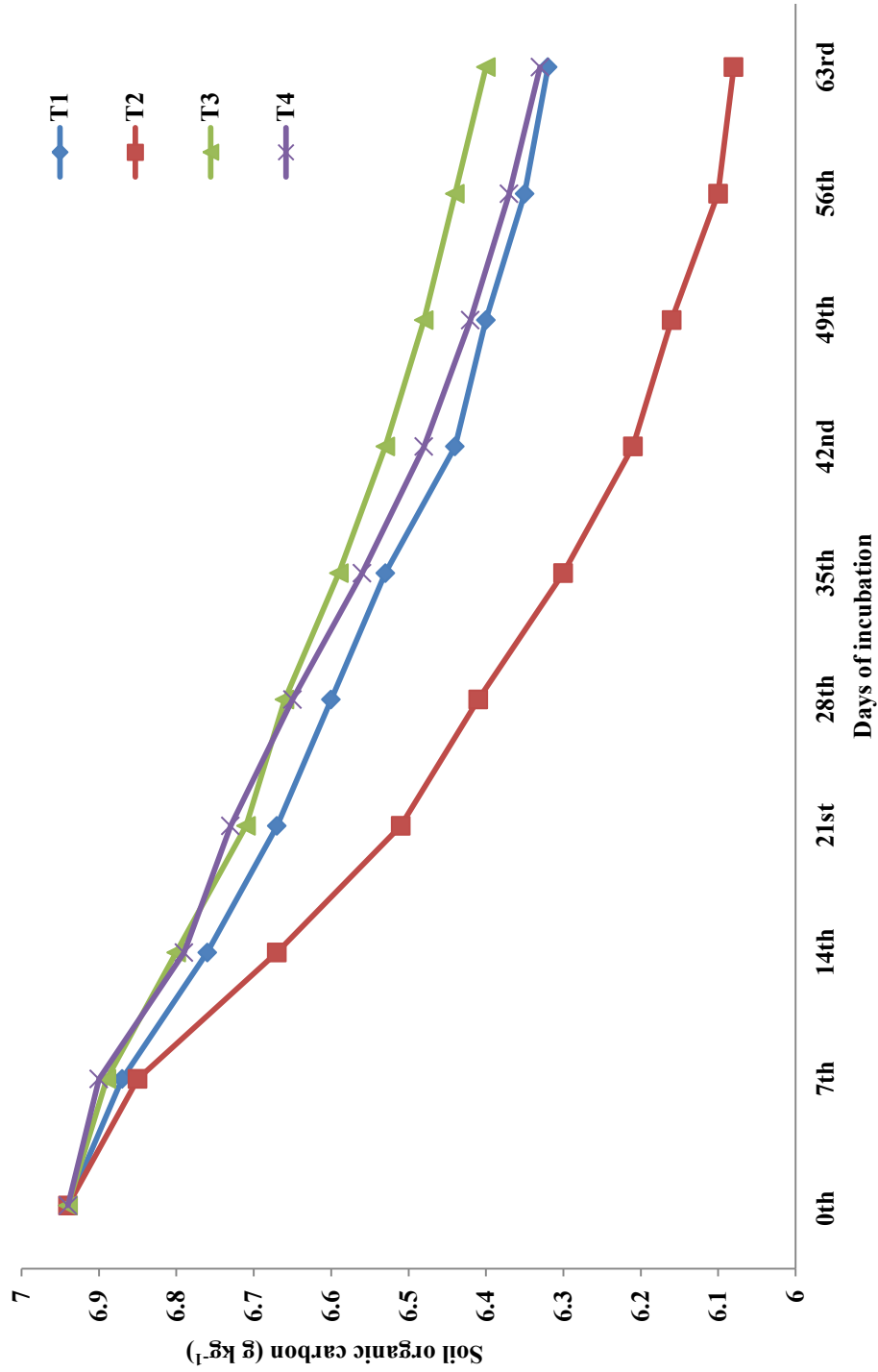


Fig. 5.4. Effect of systems of rice cultivation on soil carbon stock at harvest stage

Estimates vary, but biological activity in the floodwater is a major component of the long-term sustainability of rice systems, mainly due to the large C and N inputs associated with it (Dobermann, 2004). Primary productivity of the floodwater community ranges from about 0.2 to 2.0 g C m⁻² per day or about 10–15 per cent of that of the rice crop, resulting in large C inputs that are responsible for maintaining soil organic matter content, even without any addition of organic amendments (Bronson *et al.*, 1997; Cassman *et al.*, 1995). Moreover, algal photosynthesis contributes significant amounts of organic carbon to the soil-flood-water system (Saito and Watanabe, 1978) and a reduction in algal growth would have an impact on the carbon balance of lowland rice soils (Cassman *et al.*, 1995) and on floodwater biology. To further support the accumulation of SOC in conventional system of rice cultivation, it can be suggested that SOC is estimated by wet oxidation method which also oxidizes major portion of non-humic substances that would have directly contributed for higher accumulation of SOC.

The nitrogen fertilization caused a net decline in the soil carbon stock regardless of the systems of rice cultivation. It is obvious that addition of nitrogen might have promoted decomposition of SOC as a result of stimulated microbial growth. In order to further confirm the effect of nitrogen on SOC dynamics, an incubation study was carried out with two N levels namely with and without addition of nitrogen under two moisture regimes. Our data have corroborated with the field information that SOC declined irrespective of moisture regimes when the soil was added with nitrogen (Fig.5.5). These data strongly support that nitrogen assisted in decomposition of SOC under field conditions. Further, the correlation analysis revealed a strong negative relationship between SOC and nitrogen doses (Fig.5.6). This is in accordance with Khan *et al.* (2007) who reported a net decline in the SOC as a result of nitrogen fertilization despite massive residue C incorporation in the soil.

Overall, the data on active and passive pools of carbon clearly indicated that SRI system of rice cultivation retained higher values regardless of seasons or stages of observation. Though SRI could contribute significantly towards the accumulation of passive pools of carbon that is squarely responsible for recalcitrant carbon stock, the SOC was found to be higher in conventional system of rice cultivation. It can be inferred that SRI is beneficial in terms of passive pool of carbon which is long lasting and directly



T₁- Alternate Wetting & Drying with nitrogen; T₂- Alternate Wetting & Drying without nitrogen
T₃- Submergence with nitrogen; T₄- Submergence without nitrogen

Fig.5.5. Effect of moisture regime and nitrogen levels on the dynamics of soil organic carbon (g kg⁻¹)

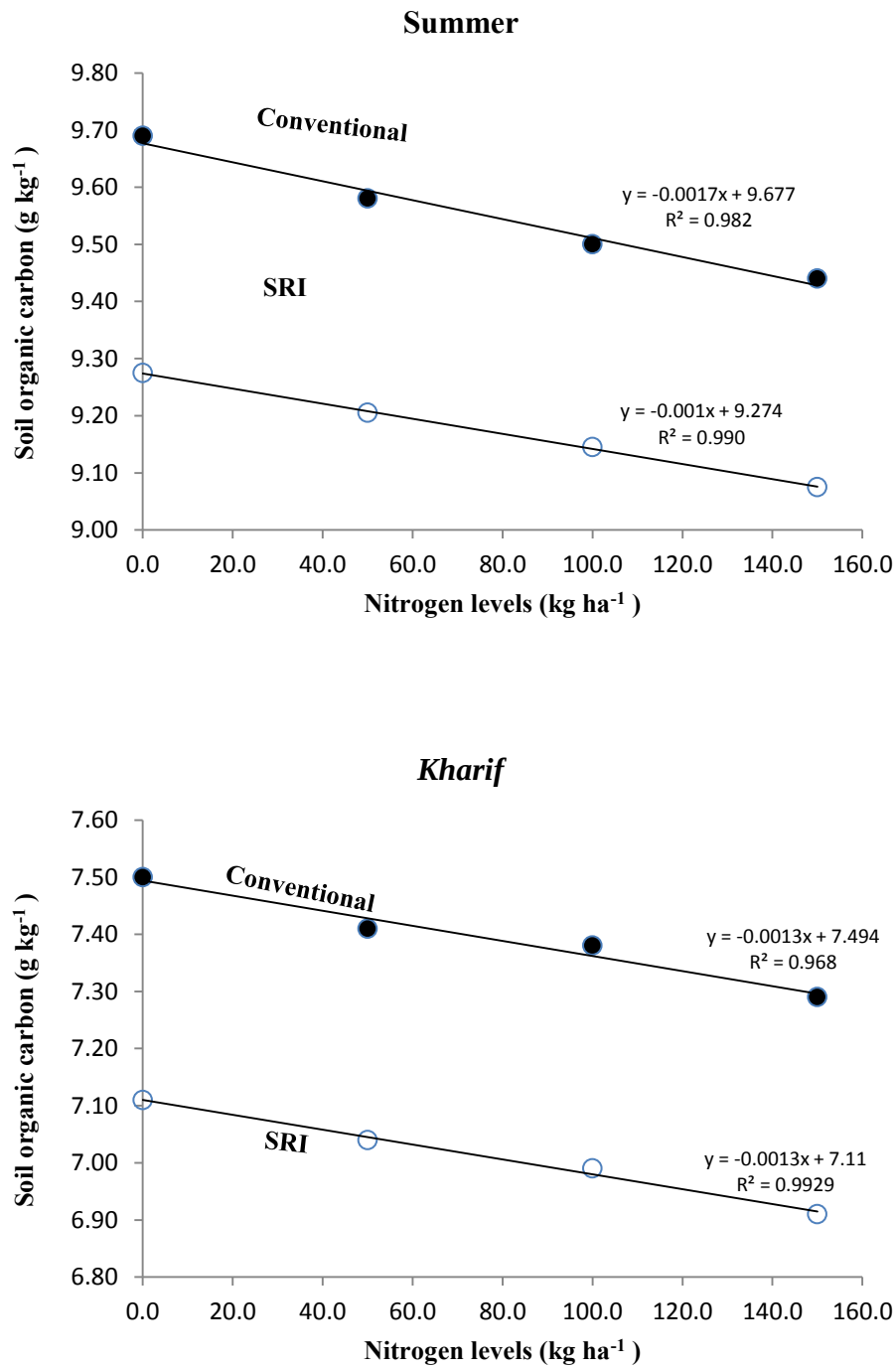


Fig 5.6. Correlation between soil organic carbon and nitrogen doses

contributes for carbon sequestration in true sense. On the other hand, the conventional system of rice cultivation had higher values of SOC stock as a consequence of continuous submergence which may or may not result as a stable pool of carbon.

5.2. Methane emission from rice fields

The South East Asian countries are often blamed for their GHG emissions particularly methane which is 25 times more potent than CO₂ in causing global warming effects. In this study, two systems of rice cultivation were examined for their methane emission with gradient levels of nitrogen. SRI system of rice cultivation carries components such as square planting, cono-weeding, alternate wetting and drying. These components favour aeration in the system while circumventing methane emission. In this study, at the early stages of rice growth we have estimated methane flux before and after each cono-weeding. Interestingly, at the beginning of the experiment before the first cono-weeding was performed, the methane emission was higher in SRI than conventional system of rice cultivation (Fig.5.7). It is plausible that SRI planting was taken up one week ahead of conventional and therefore the sequential reduction process in SRI soils might have reached methanogenesis stage and thereby contributed for higher levels of methane emission. Subsequently, our data on methane flux measured at four cycles of observations with ten days interval have clearly demonstrated that cono-weeding practice has a direct effect on reducing the methane emission in SRI (0.64, 0.59, 0.56, 0.78 mg m⁻² hr⁻¹) in comparison to its counterpart conventional system of rice cultivation (0.79, 1.37, 1.52 and 1.74 mg m⁻² hr⁻¹). The cono-weeding is an intercultural operation which stirs the soil criss-cross and that facilitates aeration and increases the Eh value (Sudhalakshmi *et al.*, 2007). Therefore, higher Eh values as a result of cono-weeding operation along with intermittent wetting and drying water management might have contributed for reduced methane emissions in SRI. This is in agreement with several reports (Conrad and Rothfuss, 1991; Hadi *et al.*, 2010; Khosa *et al.*, 2011; Li *et al.*, 2011) who have observed reduced methane flux as a result of higher Eh values facilitated by better aeration and water management strategy. Further, N fertilization has an effect on methane emission which increases with increasing levels of nitrogen. It is understandable that addition of nitrogen stimulated the methanogenic bacteria as reported in several publications (Wu *et al.*, 2009; Liang *et al.*, 2011; Datta *et al.*, 2012; Banger *et al.*, 2012). Further, the correlation analysis revealed a strong

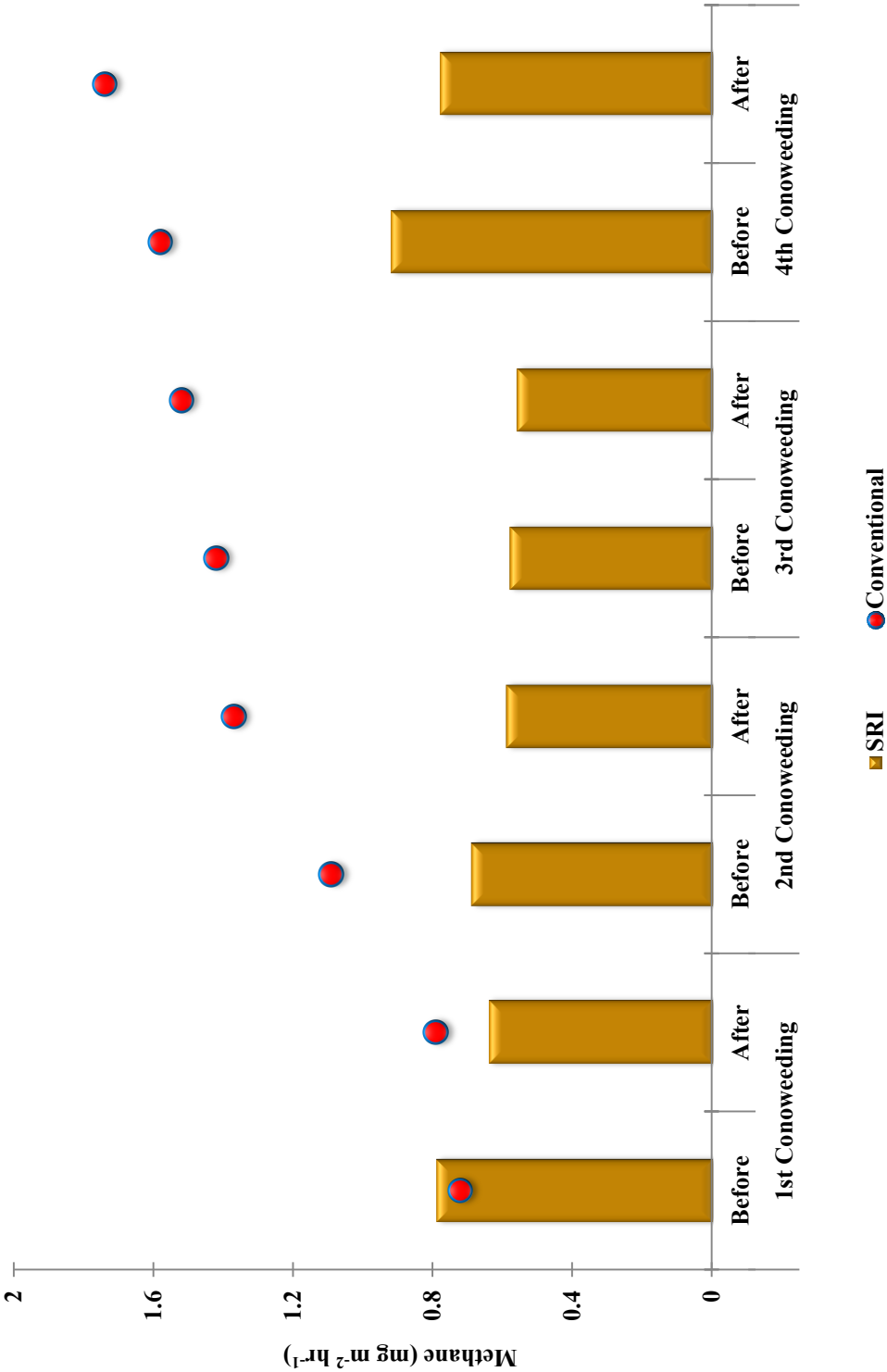


Fig.5.7. Influence of cono-weeding on methane emission rate in SRI system of rice cultivation during *kharif* season

positive relationship between methane and nitrogen doses (Fig.5.8). Despite the fact that cono-weeding practice and nitrogen application had a direct effect on decreasing or increasing methane flux in rice soils, there was no interactive effect at the initial stages of observation.

The methane flux assessment was continued till the maturity stage to gain insights of methane emission in rice soils. The data have shown that methane flux increased progressively with the advancement of crop stage, reaching the highest value at flowering and declined to the minimum at the harvest stage (Fig.5.9a and 5.9b). The data are in conformity with the findings of Aulakh *et al.* (2001b). It can be explained that the advancement of crop stages provided intense secretion of root exudates besides the rice rhizosphere is subjected to more intense reducing conditions during the flowering stage that might have favoured the activities of methanogenic bacteria and contributed for highest methane emission. This statement is being strongly supported by a number of reported literatures (Sass *et al.*, 1991; Sass and Fischer, 1996; Adhya *et al.*, 1994; Singh *et al.*, 1999; Yang and Chang, 2001, Dubey, 2005; Gogoi *et al.*, 2008). The other reason attributed for steep increase in methane emission up to flowering stage is the continuous development of aerenchyma resulting in enhanced methane transport capacity. This fact is in line with Aulakh *et al.* (2001b). Our study carries three data sets on dissolved organic carbon, methanogenic population and redox potential and the values commensurate with methane flux. The strength of the relationship with these parameters and methane flux were further confirmed by correlation co-efficient values (Fig.5.10, 5.11, 5.12). The findings are in line with reported literatures (Cheng *et al.*, 2005; Zhan *et al.*, 2011; Zschornack *et al.*, 2011; Li *et al.*, 2011). The methane flux rates were consistently lower than conventional system of rice cultivation suggesting that SRI system maintains intermittent wetting and drying and cono-weeding that facilitate aerobic oxidation while minimizing the methane emission rates. The reduced methane emission in SRI is further supported by relatively lower values of redox potential (Fig.5.13). It has been unequivocally demonstrated that methane emission rates declined with increased intensities of oxidation (Conrad and Rothfuss, 1991; Bouwman, 1991). Our observations on reduced methane emission in SRI is further supported by Ma *et al.* (2011) who indicated that alternate dry wet cycling inhibited CH₄ production because of regeneration

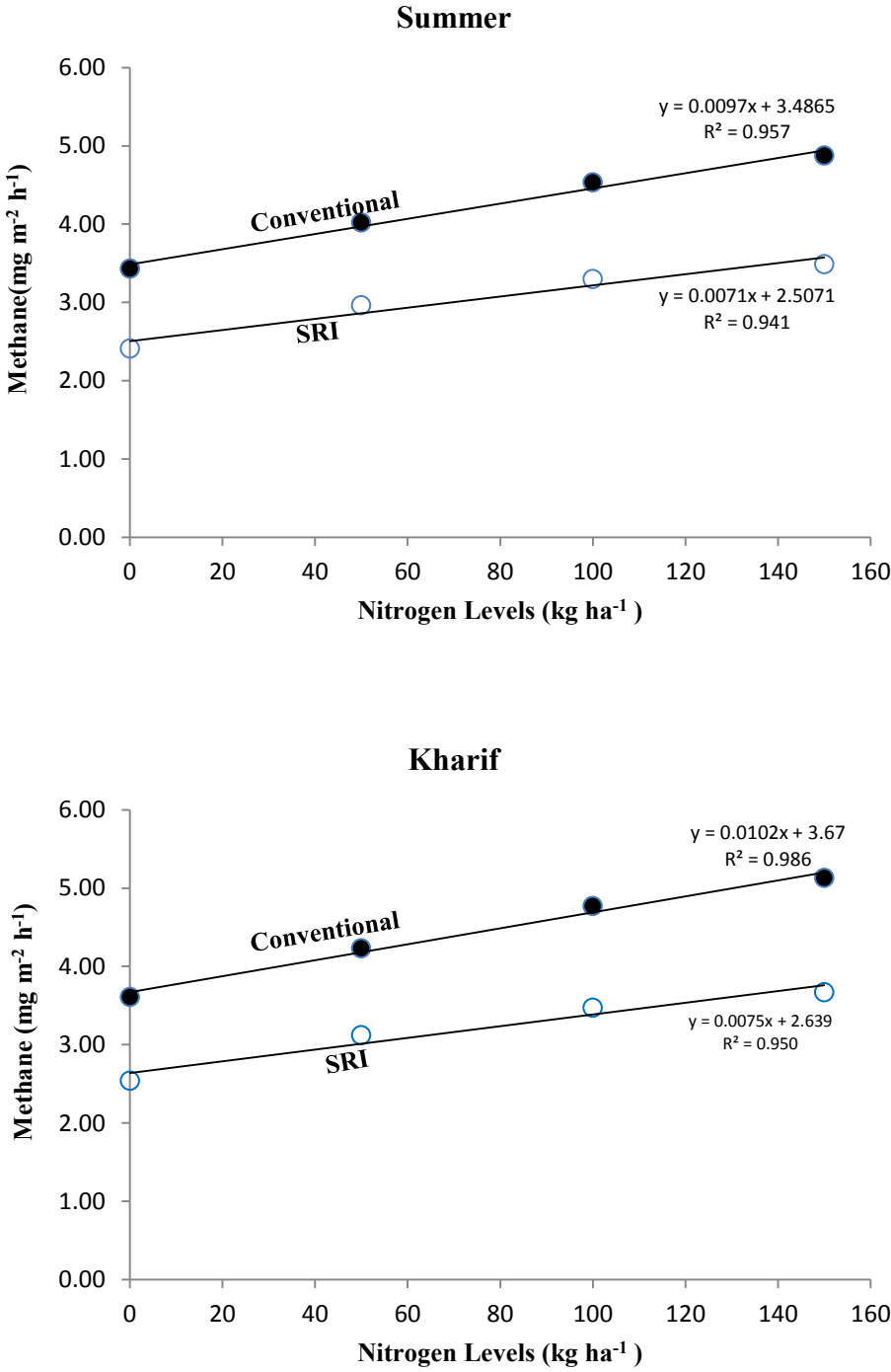


Fig 5.8. Correlation between methane emission and nitrogen doses

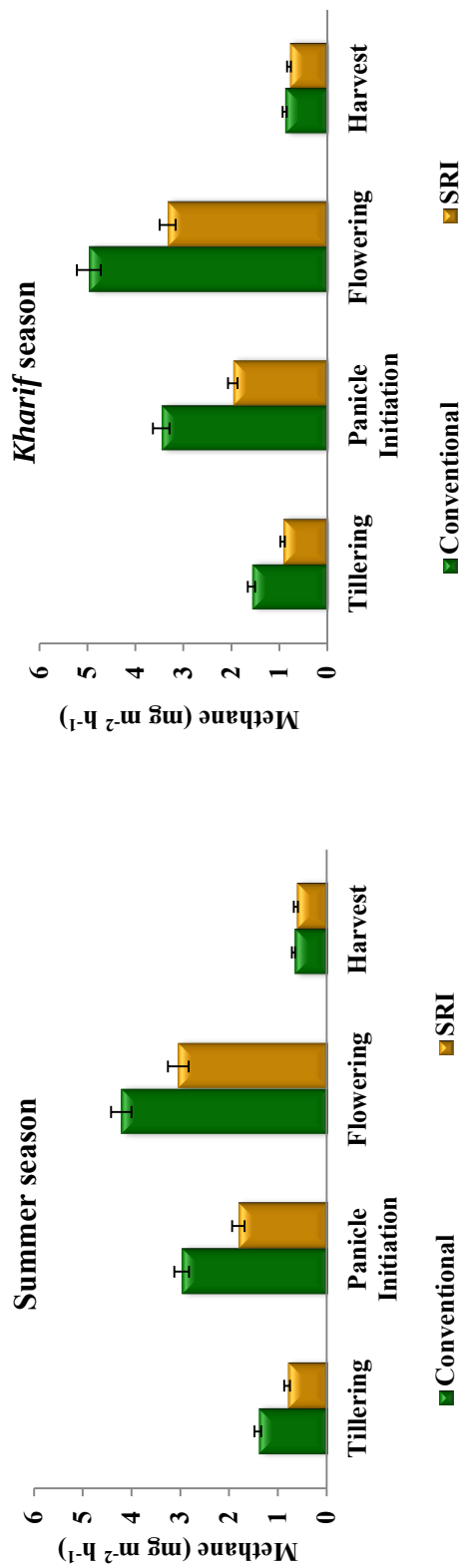


Fig.5.9a. Effect of systems of rice cultivation on methane emission at rice growth stages

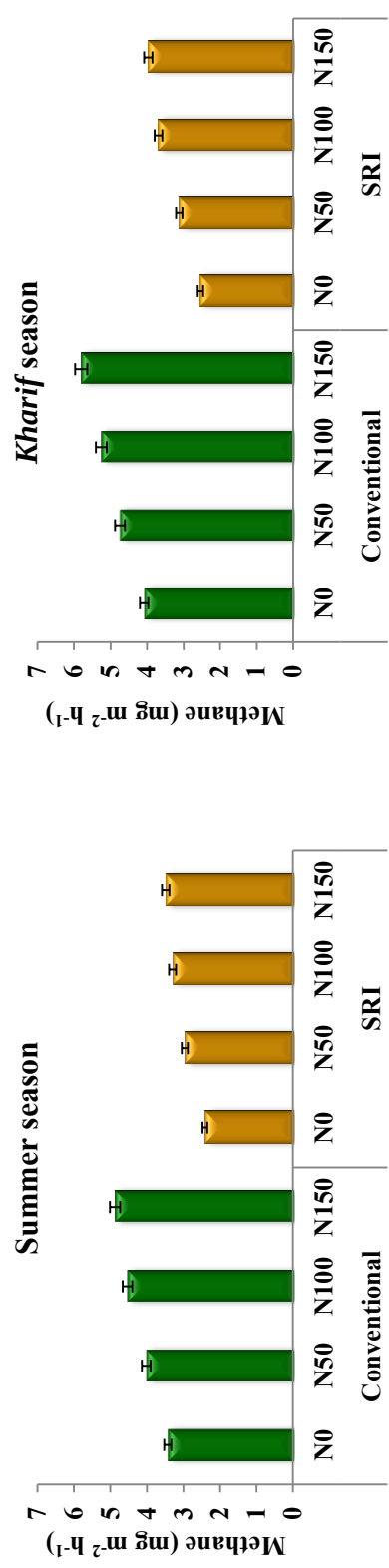
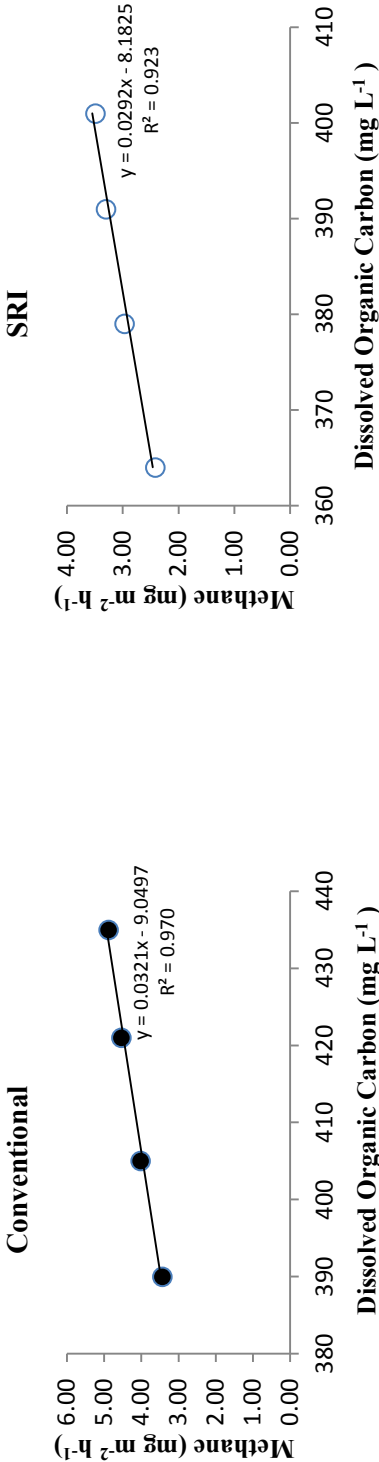


Fig.5.9b. Effect of systems of rice cultivation and nitrogen levels on methane emission at flowering stages

Summer



Kharif

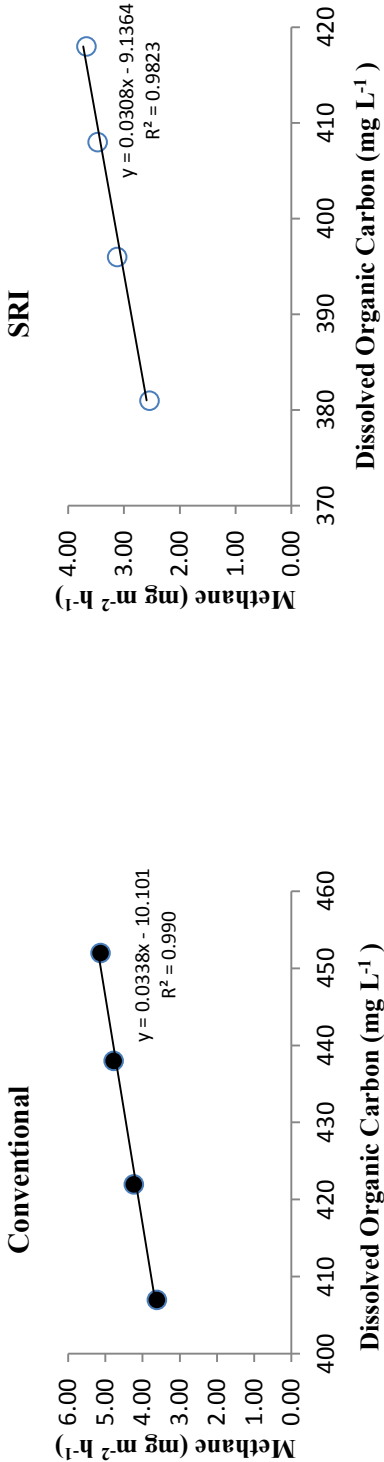


Fig 5.10. Correlation between methane emission and dissolved organic carbon (DOC)

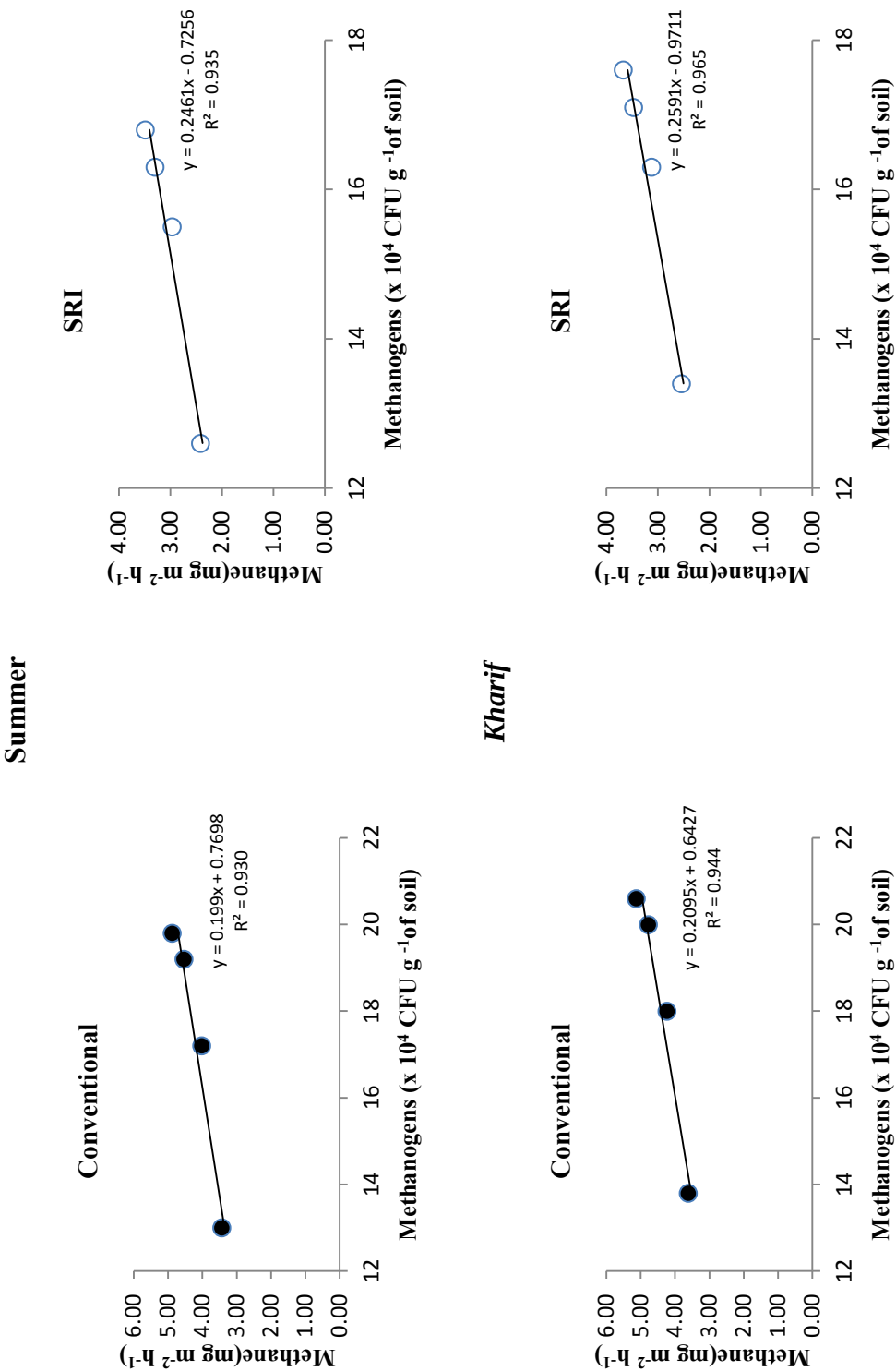


Fig 5.11. Correlation between methane emission and methanogens

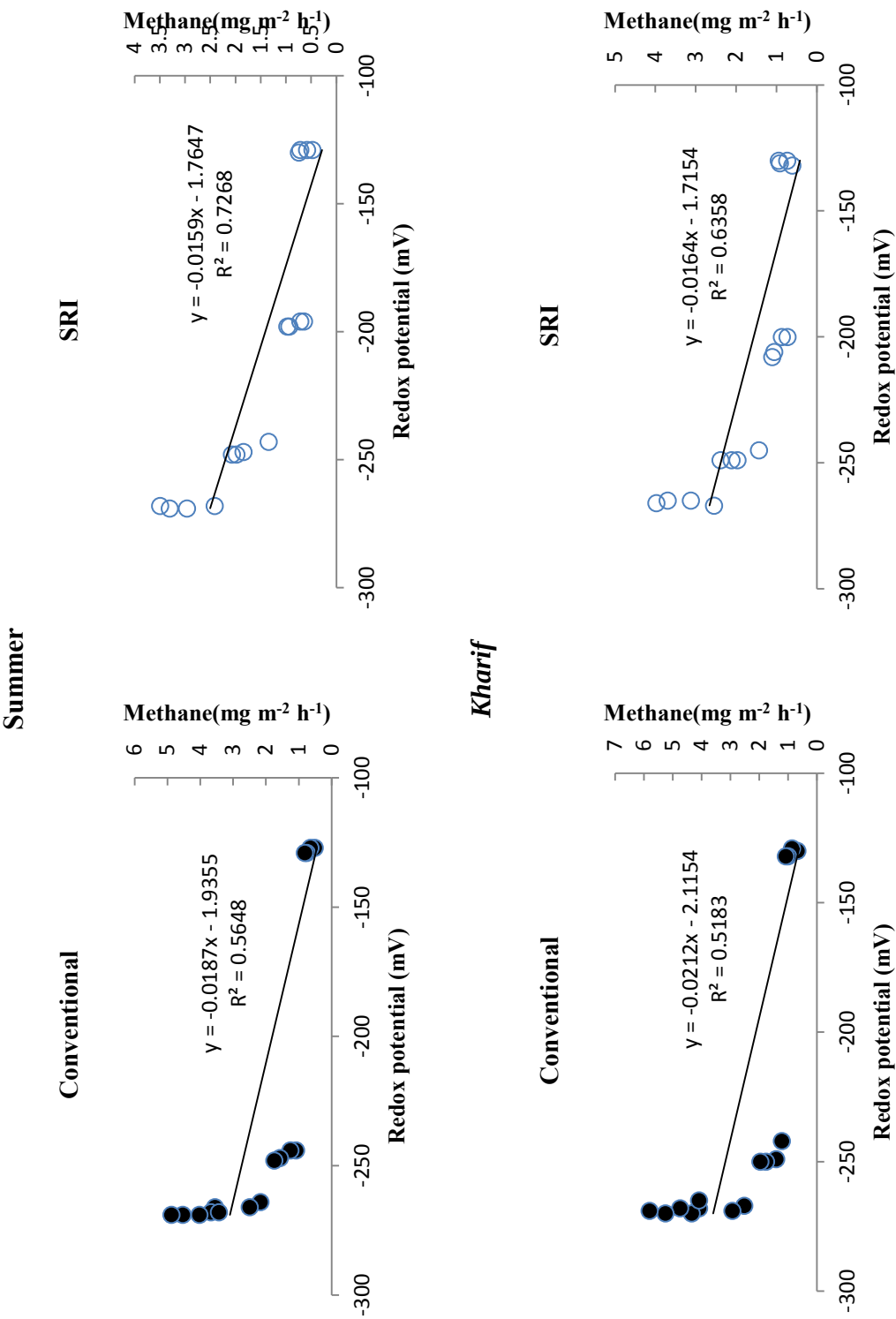
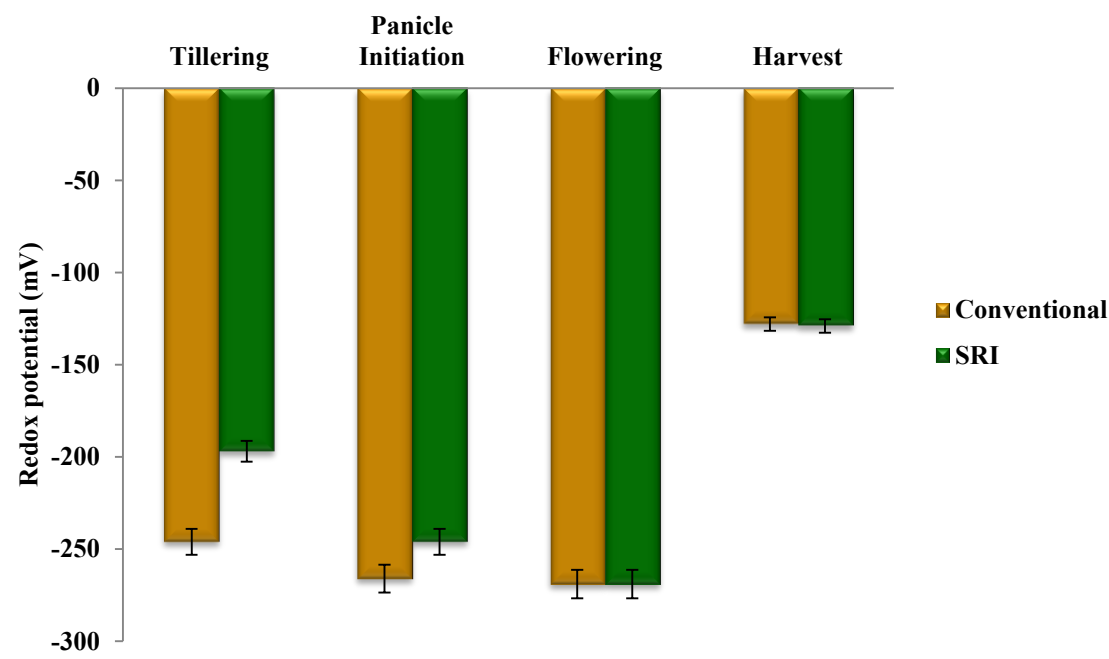


Fig 5.12. Correlation between methane and redox potential (mV)

a) Summer



b) Kharif

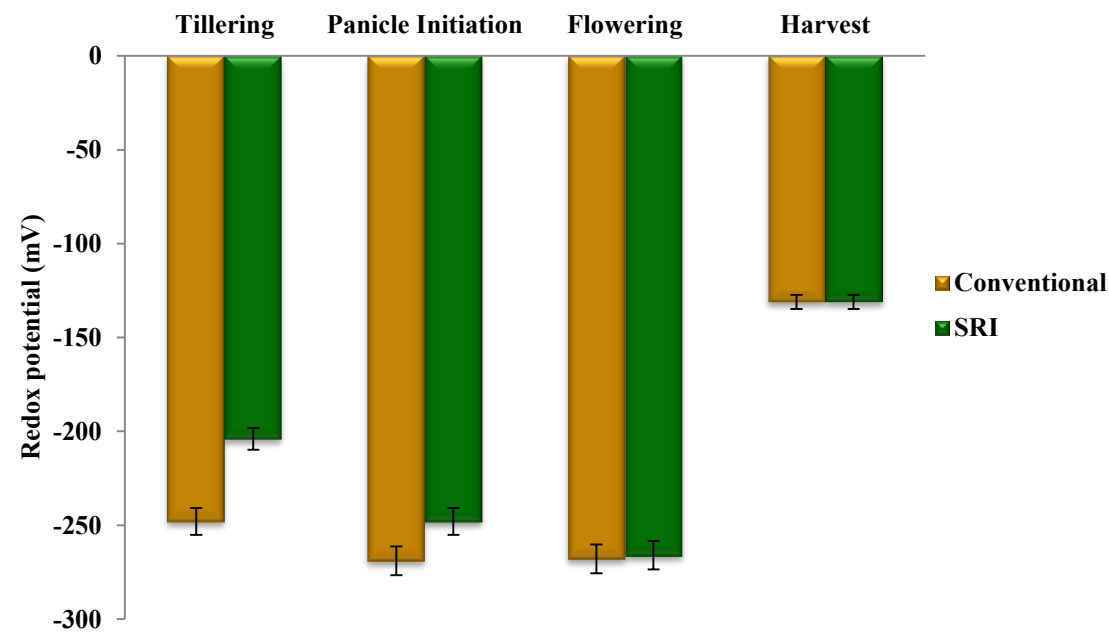


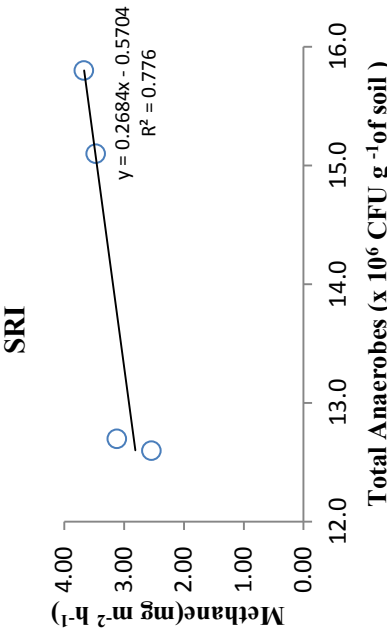
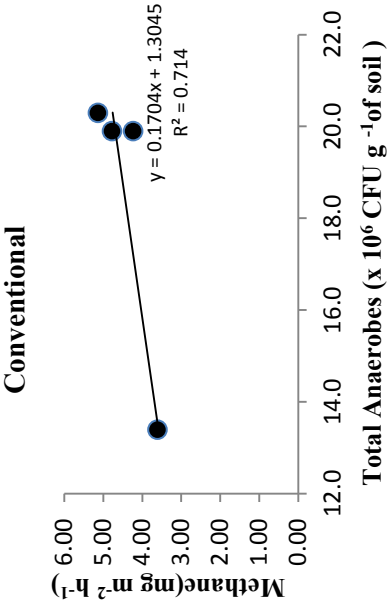
Fig. 5.13. Effect of systems of rice cultivation on soil redox potential

of Fe (III) and sulfate, which in turn allowed iron and sulfate reducing bacteria to outcompete methanogens for the substrates such as H_2 and acetate. In this study, the nitrogen fertilization significantly increased the methane emissions. This is evident that nitrogen addition increased the crop growth and probably supplied more carbon substrates for methanogens and thereby contributing for highest methane flux. This statement is in agreement with ample number of literatures (Wassmann *et al.*, 1993; Aulakh *et al.*, 2001a; Denier van der Gon *et al.*, 2002; Inubushi *et al.*, 2003). Our results are further supported by a recent meta-analysis report by Banger *et al.*, (2012) who after processing 155 data pairs from 33 published papers reported that CH_4 emissions are positively correlated with N induced crop yields and its subsequent contribution for increased carbon substrates to methanogens.

In order to understand the mechanisms associated with differential methane flux in SRI and conventional systems of rice cultivation, the microbial populations namely methanogens, methanotrophs, sulphate reducers and total anaerobes were enumerated during the course of field experimentation. Methanogens exclusively produce methane in the strict absence of free oxygen and at redox potentials of less than -150 mV. A strong positive correlation of methanogens, total anaerobes with methane emission was observed in this study (Fig.5.11 and 5.14). This study has shown that methanogenic bacterial and total anaerobes population were consistently higher in conventional system of rice cultivation than SRI (Fig.5.15a and 5.15b). Since, the conventional system has been kept under submergence for the major part of the cropping season, the lower reduction values in conjunction with dissolved organic carbon. Such changes would have favoured the methane emission in conventional system in comparison to SRI. The methanogens population has further increased by N fertilization. Many studies have clearly shown a close relationship between N fertilization and methanogenic population (Palmer and Reeve, 1993; Aulakh *et al.*, 2001b; Denier van der Gon *et al.*, 2002; Inubushi *et al.*, 2003; Banger *et al.*, 2012).

In contrast to the methanogens, the methanotrophs that are oxidizers of CH_4 which eventually decrease the level of methane flux. Consequently, the methanotrophs population was relatively higher in SRI than conventional (Fig.5.16a and 5.16b). In the present study, the correlation analysis revealed a strong negative correlation between

Summer



Kharif

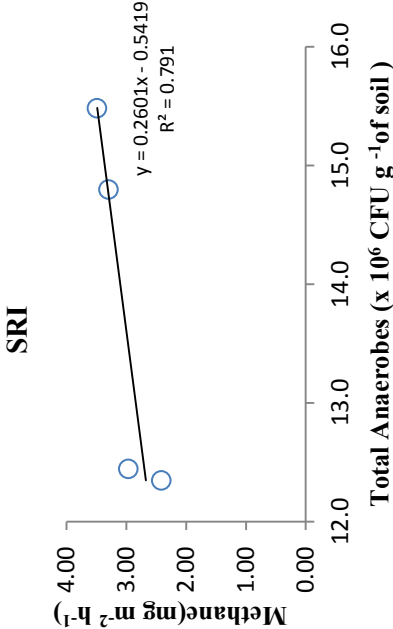
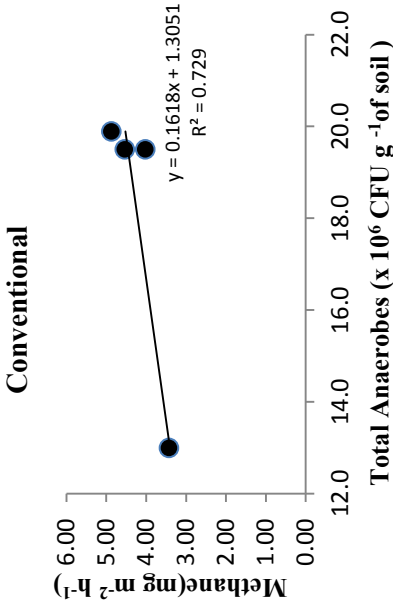


Fig 5.14. Correlation between methane emission and total anaerobes

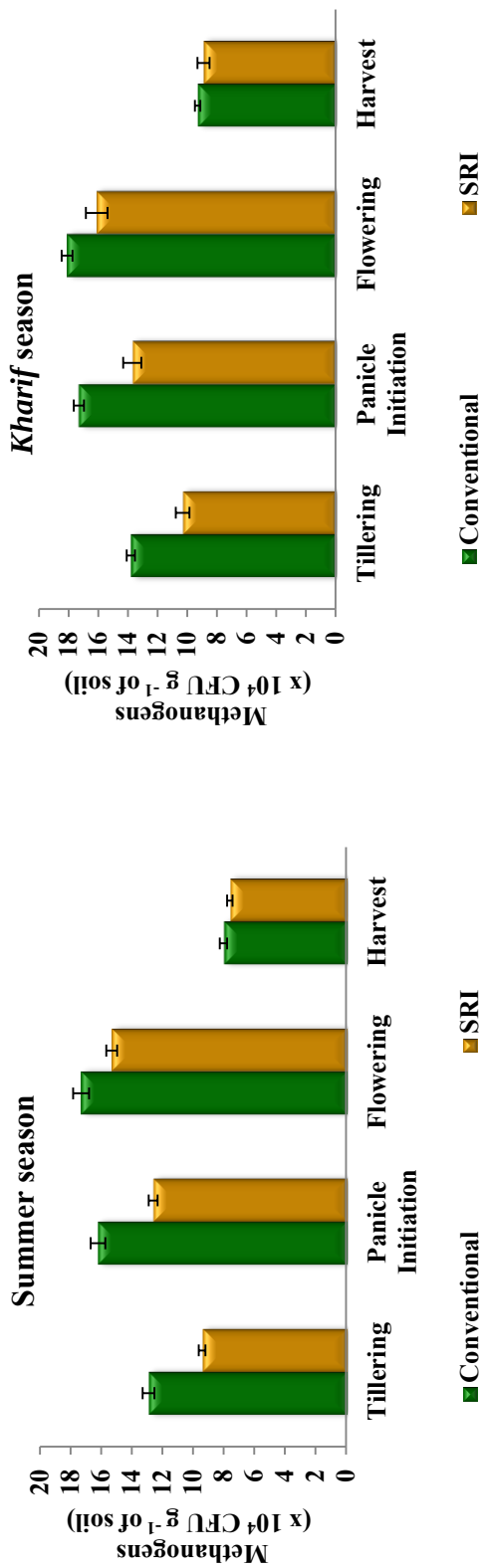


Fig.5.15a. Effect of systems of rice cultivation on methanogens at rice growth stages

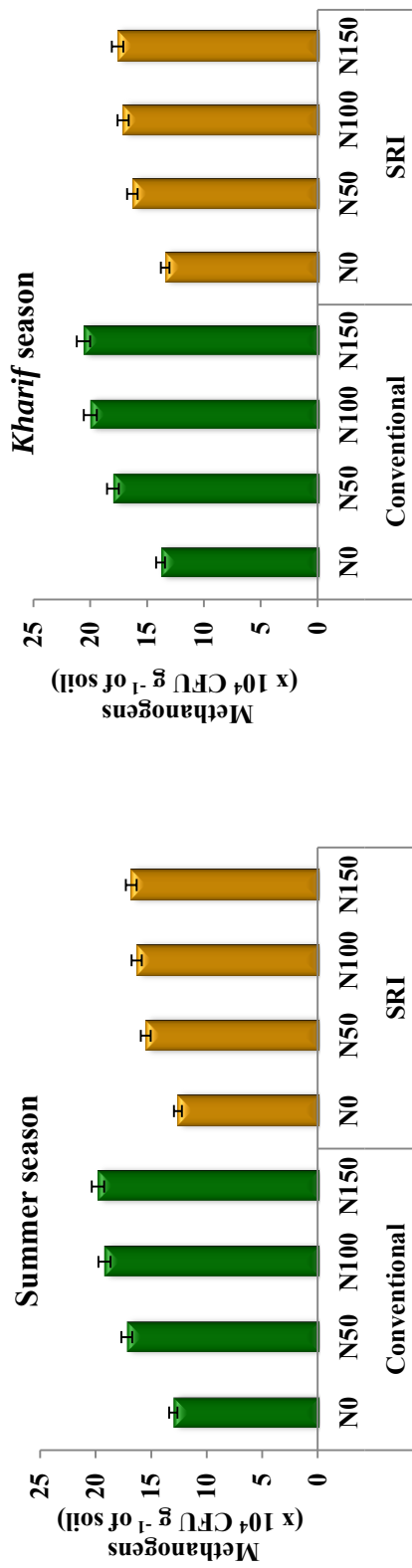


Fig.5.15b. Effect of systems of rice cultivation and nitrogen levels on methanogens at flowering stages

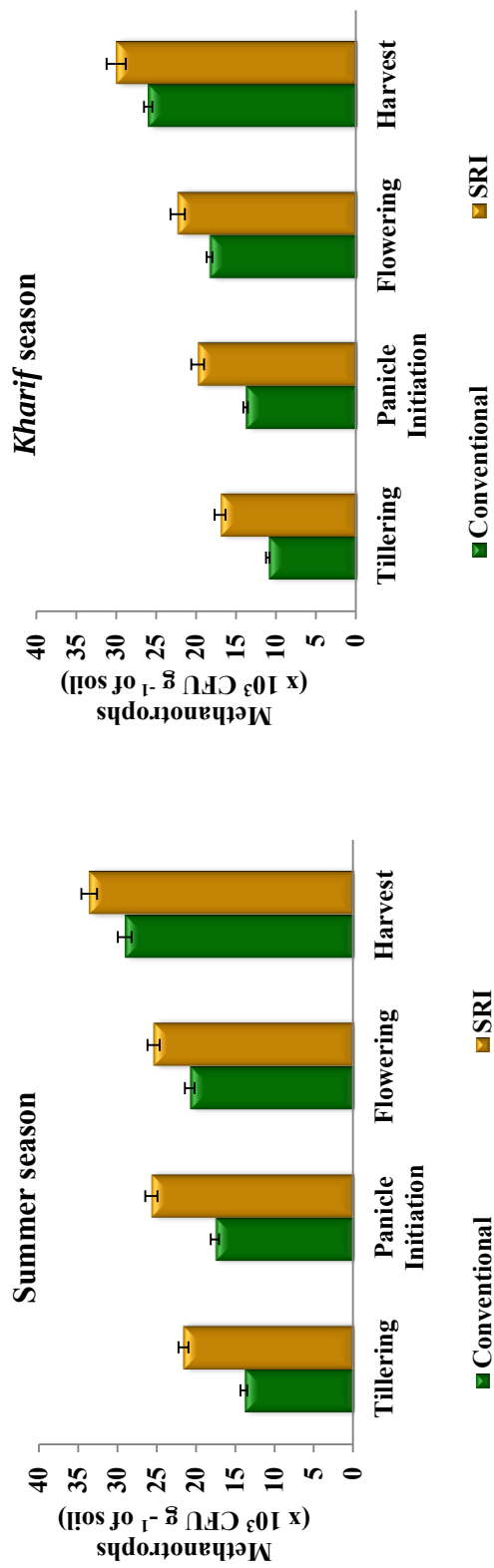


Fig.5.16a. Effect of systems of rice cultivation on methanotrophs at rice growth stages

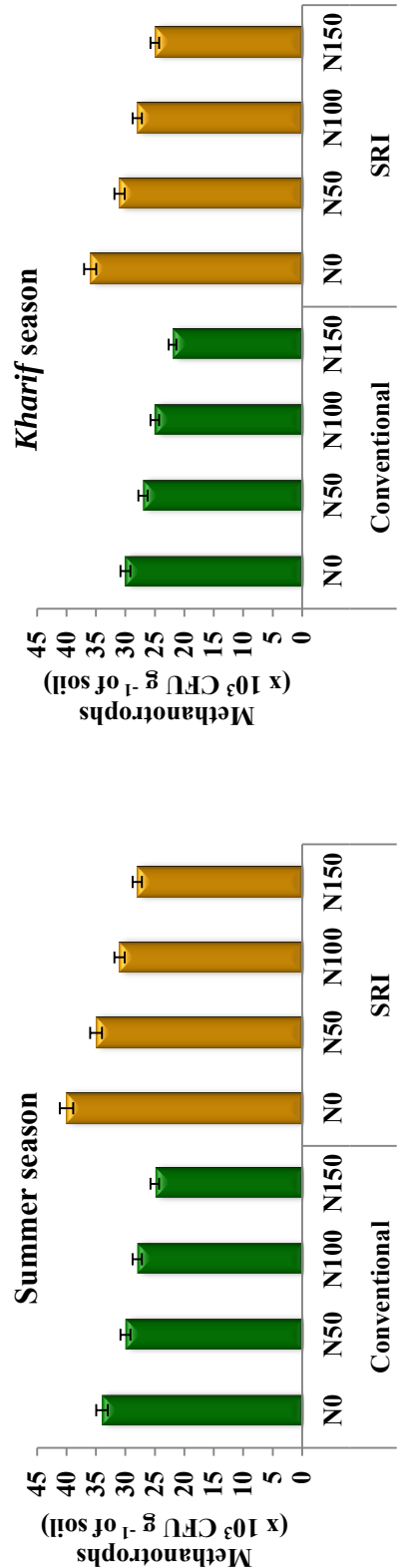
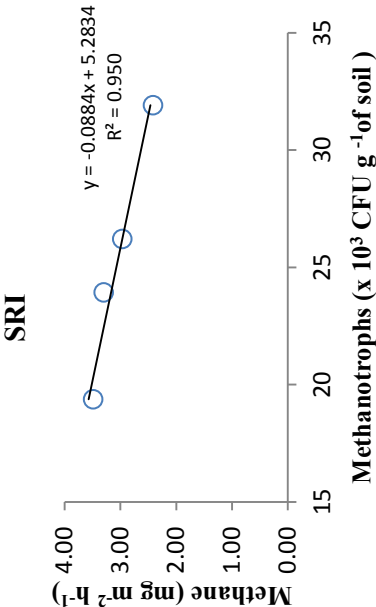
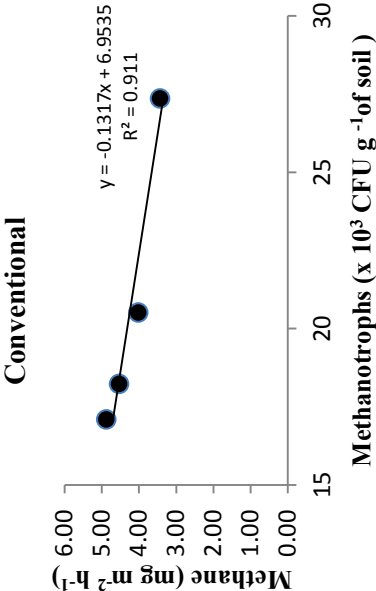


Fig.5.16b. Effect of systems of rice cultivation and nitrogen levels on methanotrophs at flowering stages

methanotrophs and methane emission (Fig.5.17). Though N fertilization stimulates the methanotrophs activities in general, under submerged soil conditions where $\text{NH}_4\text{-N}$ is predominant, it has an inhibitory effect on methanotrophs. The CH_4 and NH_4 molecules have similar size and structure and therefore methane monooxygenase (MMO) enzymes of methanotrophs oxidize NH_4 instead of CH_4 and thereby inhibit CH_4 consumption (Dunfield and Knowles, 1995; Hanson and Hanson, 1996; Dubey, 2005). Furthermore, the intermediates and end-products of ammonia oxidation, i.e. hydroxylamine and nitrite are considered to be toxic for methanotrophs (Schimel, 2000; Singh, 2011). This statement is strengthened by our correlation data showing strong negative correlation of $\text{NH}_4\text{-N}$ with methanotrophs population (Fig.5.18). So far many mechanisms have been proposed for stimulation and inhibition effects on methane oxidation and methanotrophs, but none of them has yet been experimentally verified under field conditions (Singh, 2011). However, our results have contributed important information on the effects of nitrogen fertilization on methanotrophs under field level.

The sulphate reducing bacterial population dynamics varied widely between SRI and conventional systems of rice cultivation. Though methanogens and sulphate reducing bacteria are reported to be inversely related, the present study brings out a new information indicating variability in microbial activities with the stage of the crop. The activities of sulphate reducing bacteria at the tillering phase in conventional was very intense due to the fact that this system is submerged in combination with lower Eh values. These situations favour the activities of sulphate reducing bacteria. Such response vitiated during the later phase of the crop growth. The reverse was true with SRI where sulphate reducing bacterial activities were higher than conventional but progressively decreased with the age of the crop. The diminishing trend of sulphate reducing bacteria is due to the competition with methanogens. Previous studies have reported that sulphate reducing bacteria suppress CH_4 production in rice soils but do not fully out-compete the methanogens for substrate (van Bodegom and Stams, 1999; Denier van der Gon *et al.*, 2001). The correlation analysis revealed a strong negative correlation between sulphate reducing bacteria and methane emission (Fig.5.19).

Summer



Kharif

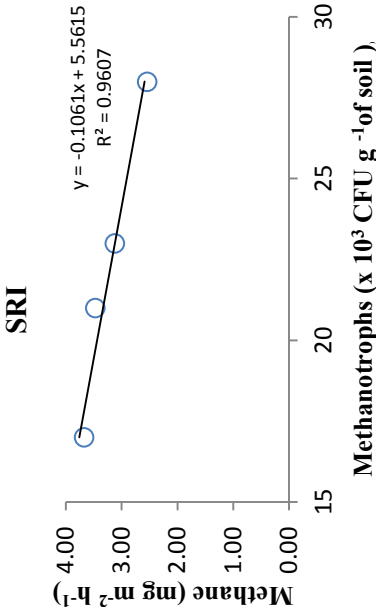
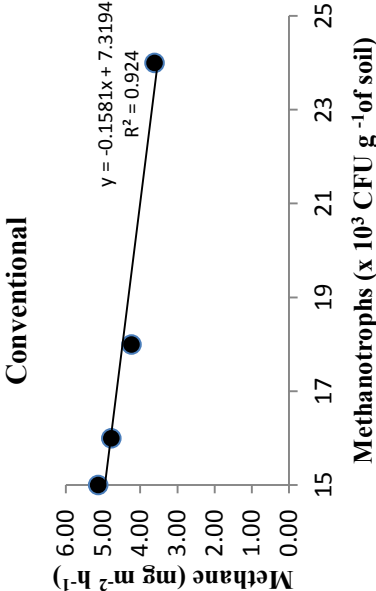


Fig 5.17. Correlation between methane emission and methanotrophs

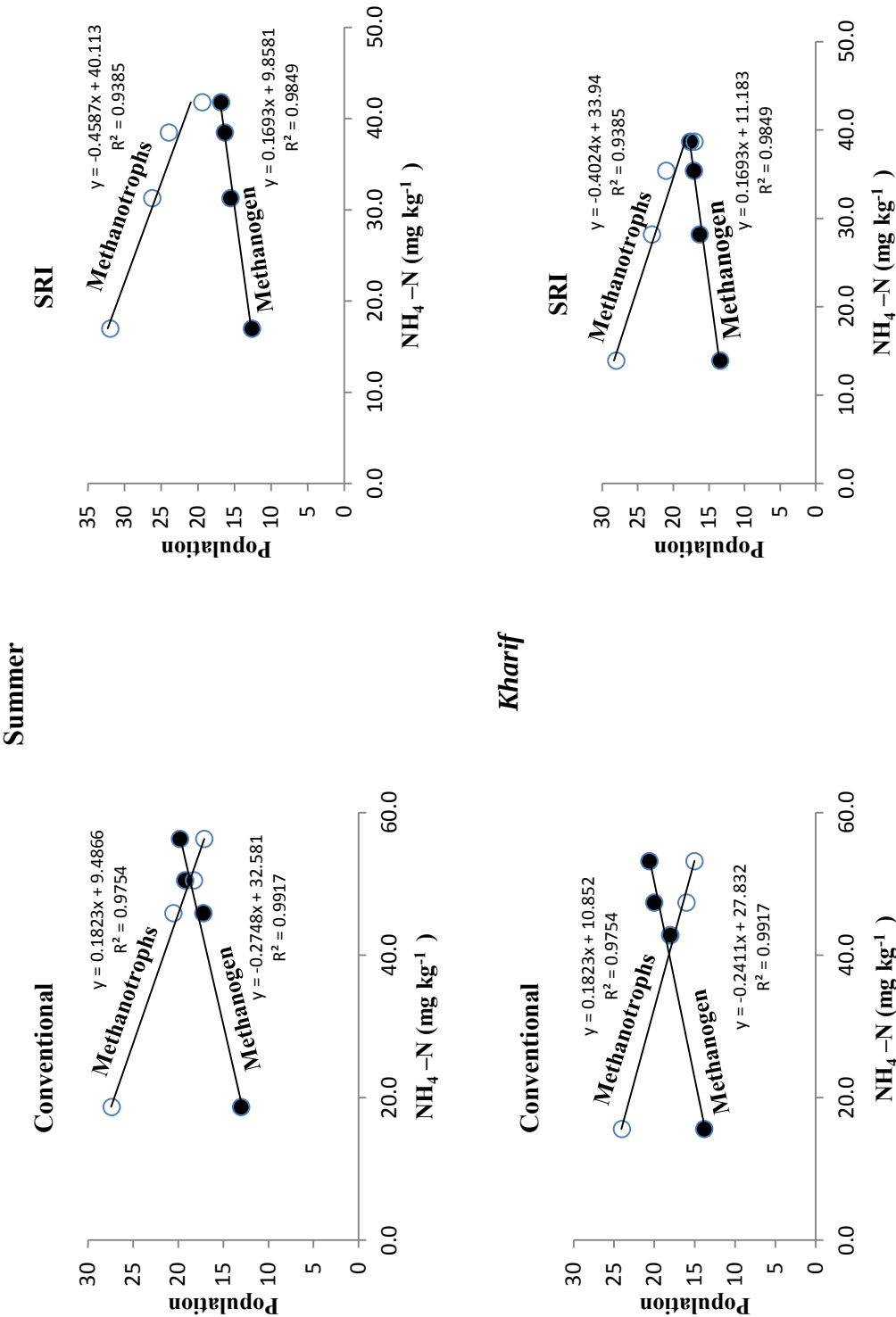
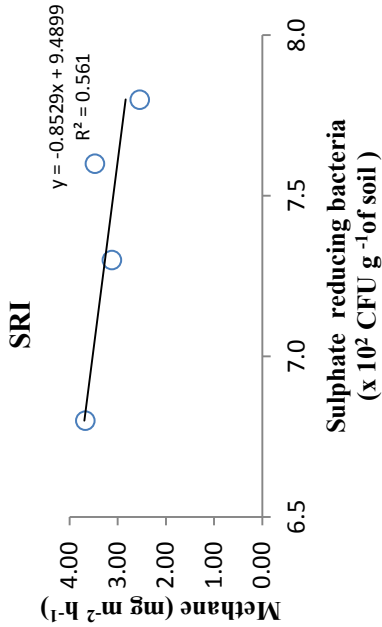
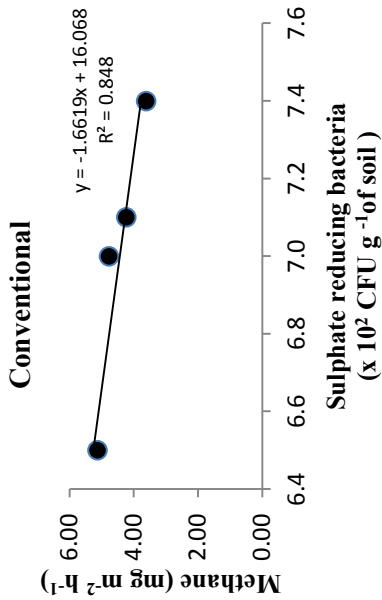


Fig 5.18. Correlation of methanogens, methanotrophs with $\text{NH}_4\text{-N}$ concentration (mg kg⁻¹)

Summer



Kharif

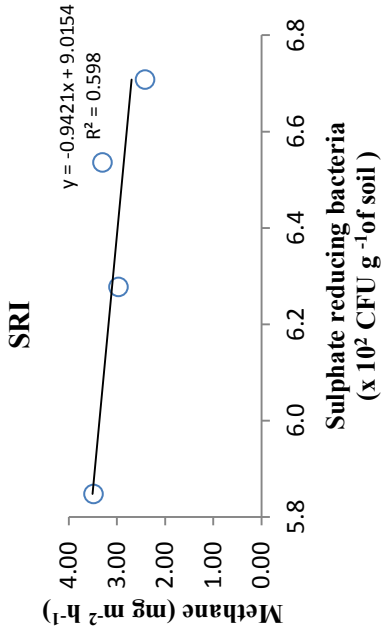
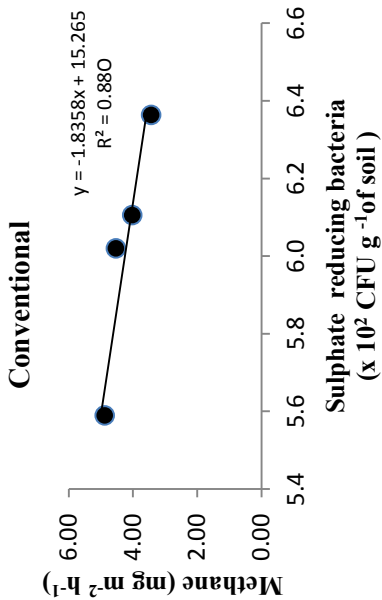


Fig 5.19. Correlation between methane and sulphate reducing bacteria

5.3. Carbon dioxide emission from rice fields

In the present investigation, the carbon di oxide emission was estimated in two distinct systems of rice cultivation at varying levels of nitrogen. The measurements at four critical stages of rice growth revealed that the CO₂ emission was significantly higher in SRI than conventional system of rice cultivation (Fig.5.20a and 5.20b). It is obvious from the fact that in SRI system, the alternate wetting and drying might have influenced the rapid decomposition process by triggering intense microbial activities and thereby contributed for higher CO₂ emissions. This statement is strengthened by our correlation data showing positive correlation of SMBC, bacterial population and dehydrogenase activities with CO₂ emission (Fig.5.21, 5.22, 5.23).

Carbon dioxide is released from soil through respiration that includes three biological processes namely, microbial respiration, root respiration and faunal respiration (De-Jong, 1974; Jorgensen, 1973; Edward, 1975) and one non-biological process, *i.e* chemical oxidation (Bunt and Rovira, 1954). Soil microflora contributes 99 per cent of the CO₂ emitted as a result of decomposition of organic matter, while the contribution of soil fauna is much less (Rastogi *et al.*, 2002). These facts strengthens our findings that SRI soils with intense microbial populations throughout the cropping period might have directly contributed for higher CO₂ emission in comparison to conventional system.

One of the most important factors in SRI contributing for higher CO₂ emissions is the intermittent wetting and drying style of water management. Several studies (Orchard and Cook, 1983; Rastogi *et al.*, 2002) have reported that the intermittent wetting and drying of soil has a pronounced influence on CO₂ evolution. When the soil is rewetted, the activity of the microbes increases accompanied by release of air trapped in the soil pores contributing to an increase in CO₂ emission. Our data have shown that CO₂ emission in SRI increased by 82.2 and 53.7 per cent over conventional system in summer and *kharif* seasons, respectively, during active tillering stage where intermittent wetting and drying irrigation management was practiced. Similar results were reported by Borken *et al.* (1999) who observed that rewetting of soils increased the CO₂ emissions by 48 to 144 per cent.

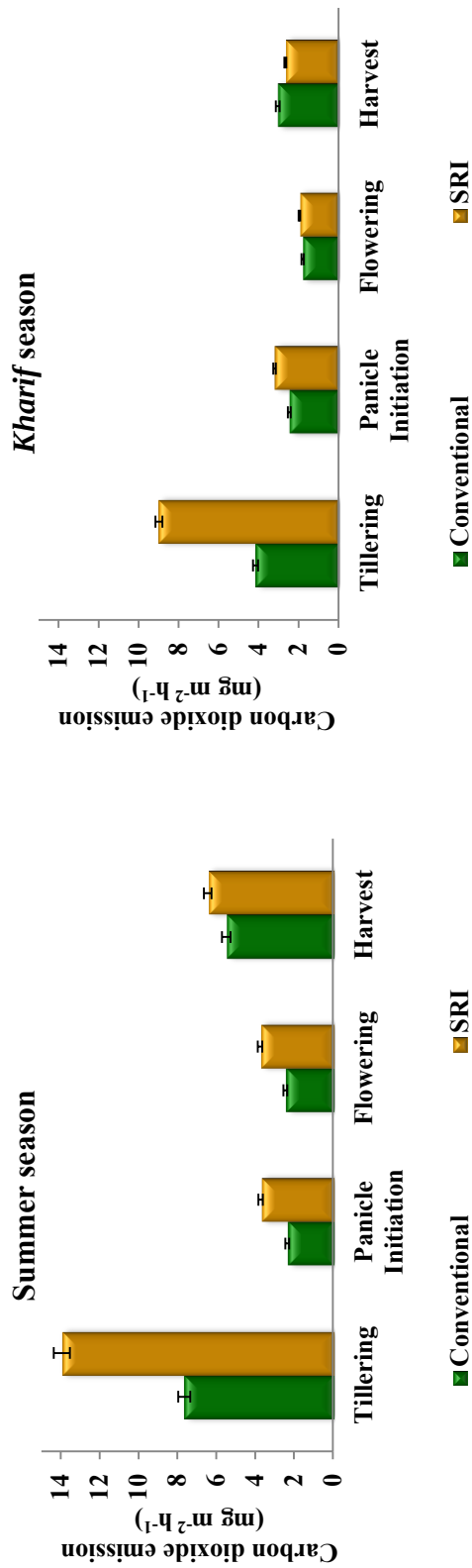


Fig.5.20a. Effect of systems of rice cultivation on carbon dioxide emission at rice growth stages

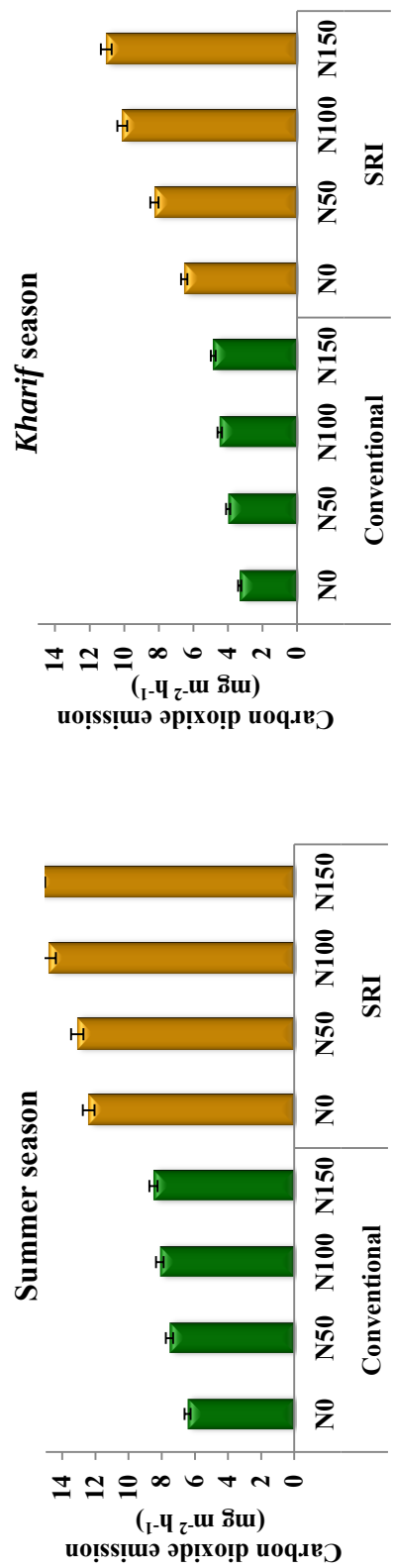


Fig.5.20b. Effect of systems of rice cultivation and nitrogen levels on carbon dioxide emission at tillering stages

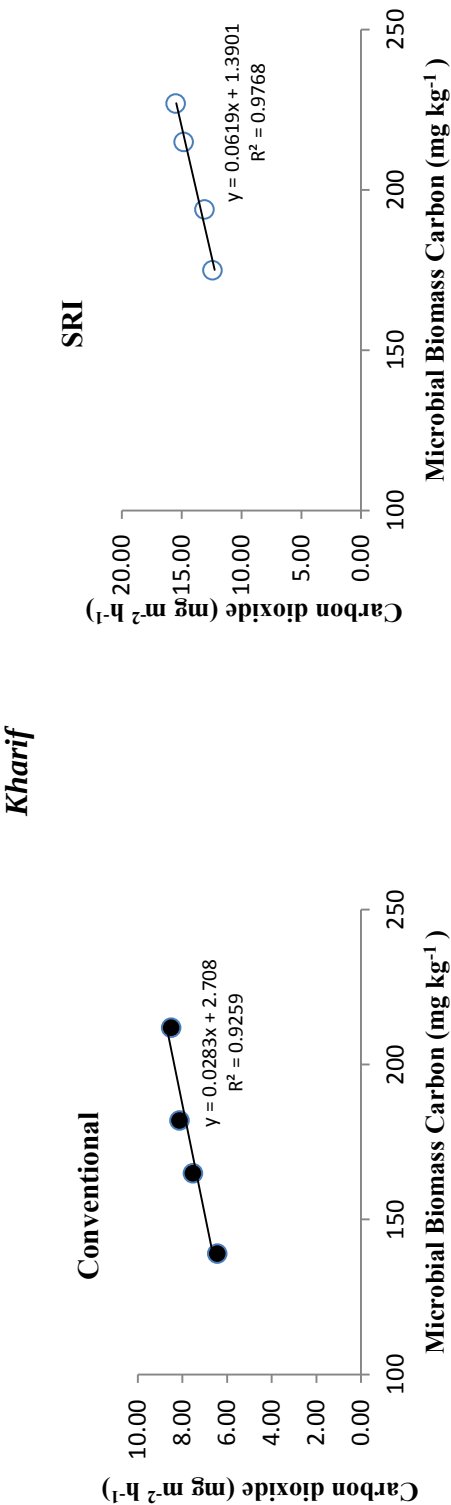
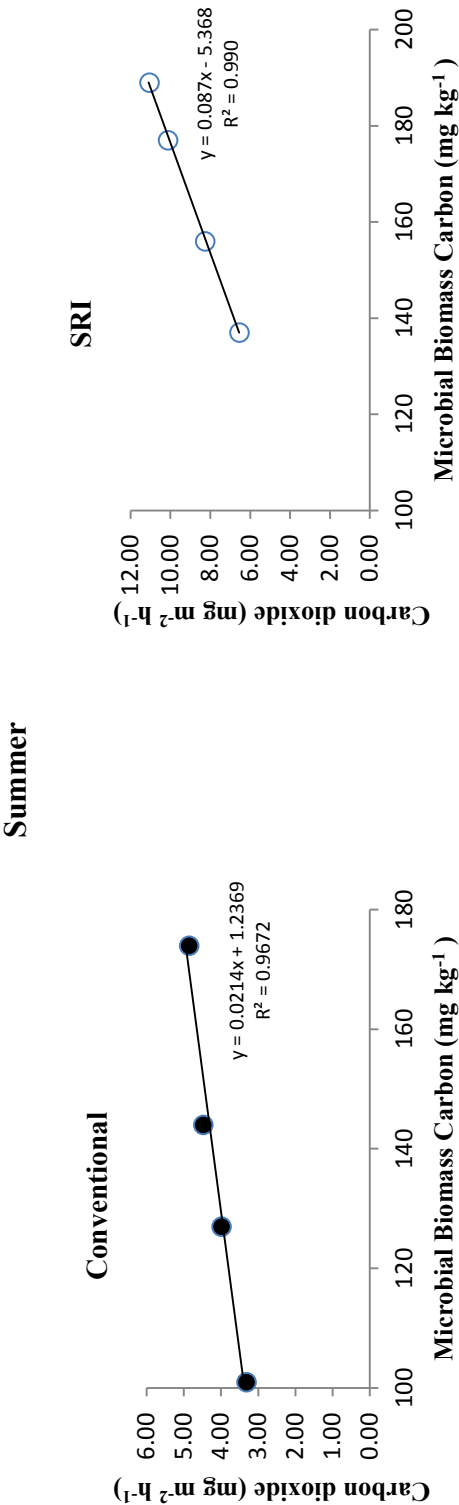


Fig 5.21. Correlation between CO₂ emission and microbial biomass carbon

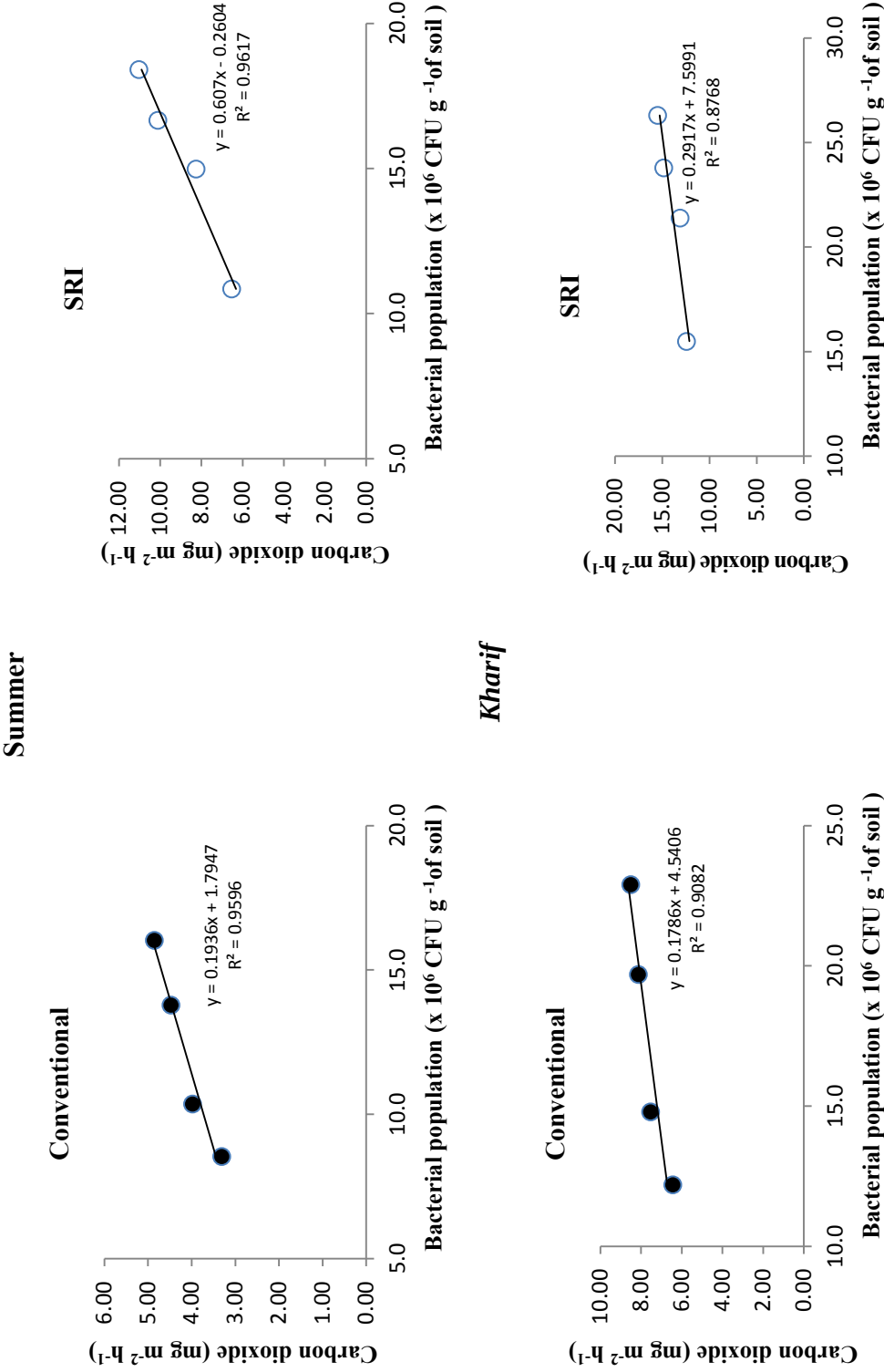
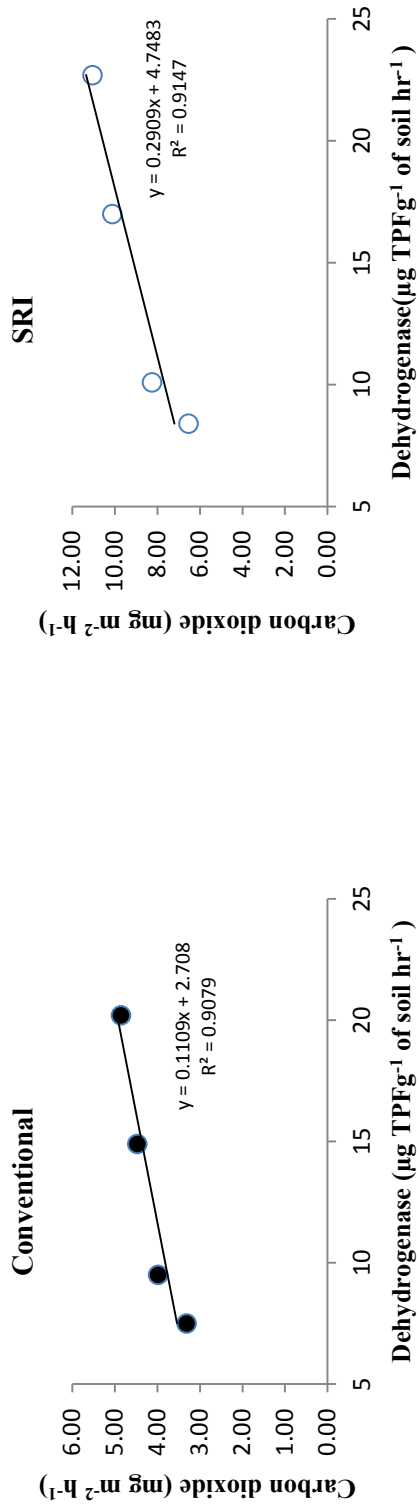


Fig 5.22. Correlation between CO₂ emission and bacterial population

Summer



Kharif

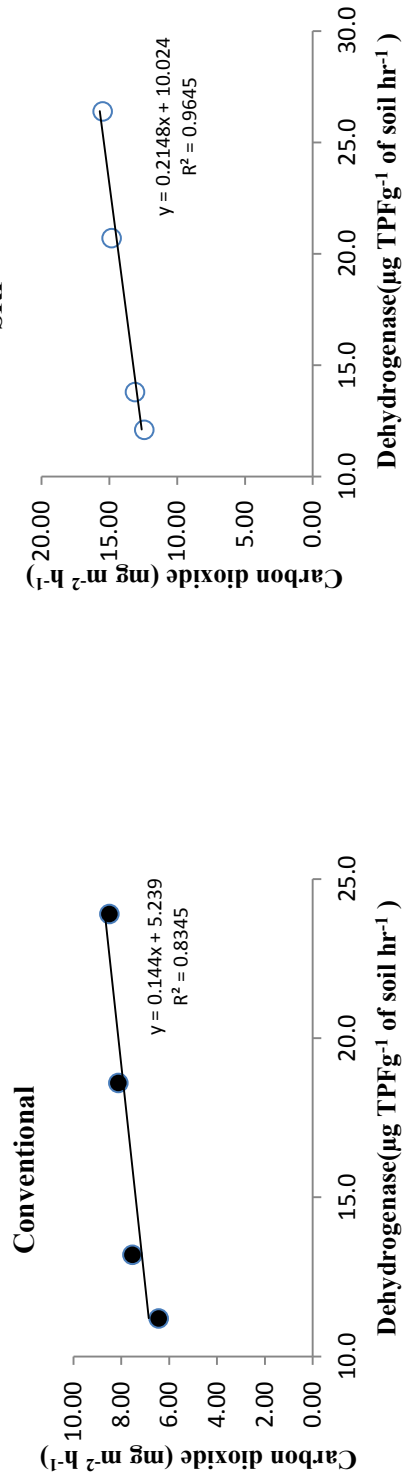


Fig 5.23. Correlation between CO₂ emission and dehydrogenase activities

In order to support the positive relationship between alternate wetting and drying with higher CO₂ evolution, we conducted incubation study with two levels of moisture regimes simulating the field conditions. Our data have corroborated with the information that the soil when kept under alternate wetting and drying conditions emitted higher CO₂ in comparison to continuous submergence. Under intermittent wetting and drying conditions, the decomposition of SOM is accomplished by a large group of microorganisms. Owing to the high energy release associated with aerobic respiration of these organisms, decomposition of SOM and synthesis of cell substances proceed rapidly resulting in the production of large amounts of CO₂. Under submerged conditions, the decomposition of SOM is carried out by facultative and obligate anaerobes. Since anaerobic bacteria operate at a much lower energy level than aerobic, both decomposition and assimilation are slower in soil under submergence (Ponnamperuma, 1972; Sahrawat, 2005). The introduction of aerobic phases in lowland rice systems has been found to result in decreased organic C (Witt *et al.*, 2000). Under aerobic conditions, most of the C entering the soil is labile and therefore, respired back to the atmosphere. These findings strongly support our explanation that the alternate wetting and drying water regime contributed for higher CO₂ emission in SRI under field conditions.

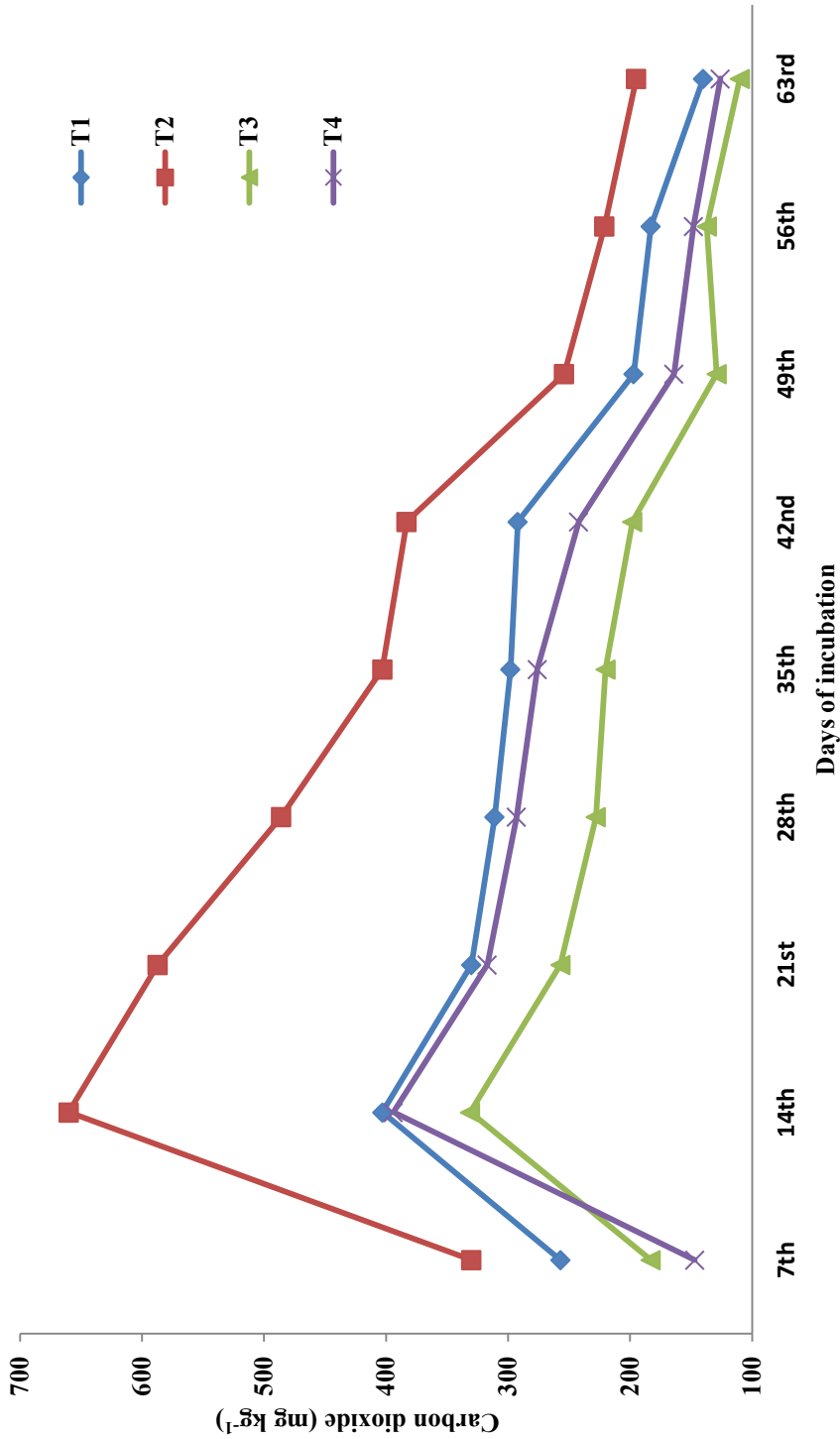
The other critical factor in SRI that might have influenced higher CO₂ emissions is the four times of cono-weeding operation. Studies have revealed that soil disturbances such as tillage breaks down soil aggregates, exposes SOC, produces a soil microenvironment favourable for accelerated microbial decomposition besides facilitating for chemical oxidation and thereby increases CO₂ production (Doran *et al.*, 1994; Kessavalou *et al.*, 1998; Ball *et al.*, 1999).

In the case of conventional system, the reduced CO₂ emission is evident from the fact that this system of rice cultivation practices continuous submergence. In flooded fields, soil CO₂ efflux is reduced due to limitation of diffusion of oxygen and suppression of CO₂ emissions (Alberto *et al.*, 2009). The presence of floodwater prevents aerobic decomposition to occur and it also serves as a barrier for CO₂ diffusion to the atmosphere (Miyata *et al.*, 2000).

Our field data on CO₂ emissions also indicated that the CO₂ emission rate was highest during early stages of crop growth. This is also attributed for differences in the water management (Alberto *et al.*, 2009) besides the energy source for decomposing microorganisms may be higher and active at early stages. This statement is strengthened by our data on bacterial, fungal, actinomycetes population besides microbial biomass carbon which commensurate with CO₂ emissions.

The results of the field experiments have shown that nitrogen fertilization significantly increased the CO₂ emissions by 27 to 40 per cent over the control. This is evident that the addition of nitrogen stimulated the soil microorganisms and thereby increased the CO₂ emissions. This is in accordance with various studies (WildDung *et al.*, 1975; Bunnell, 1977; Xiao *et al.*, 2005; Iqbal *et al.*, 2009) which reported that N fertilization significantly increased the CO₂ flux. N fertilization enhanced the CO₂ emissions in rice fields and this may be attributed to the efficient use of C for microbial growth (Fisk and Fahey, 2001) and/or microbial biomass assimilates carbon less efficiently (Anderson, 1994). In order to confirm the effect of nitrogen on CO₂ emission, we conducted a laboratory incubation experiment and the results revealed that the nitrogen addition produced more CO₂ as a result of enhanced SOC decomposition irrespective of moisture regimes (Fig.5.24). Further, the correlation analysis revealed a strong positive relationship between CO₂ emission and nitrogen doses (Fig.5.25). These data strongly support that nitrogen contributed for higher CO₂ emission under field conditions.

Field experiments in two seasons have clearly indicated the seasonal variability of CO₂ emissions irrespective of the systems of rice cultivation. The total CO₂ emission in summer season was increased by 51 per cent over the *kharif* season. This may be attributed to the fact that the higher temperature in summer season might directly contribute for rapid decomposition of SOC and in turn increased the CO₂ emissions. Many authors have observed that the temperature has a marked effect on CO₂ evolution from the soil (Bridgham and Richardson, 1992; Chapman and Thurlow, 1998; Rastogi *et al.*, 2002). Our data are further supported by Wiant (1967) who observed a logarithmic increase in CO₂ evolution between 20 and 40°C.



T₁- Alternate Wetting & Drying without nitrogen; T₂- Alternate Wetting & Drying with nitrogen
T₃- Submergence without nitrogen; T₄- Submergence with nitrogen

Fig.5.24. Effect of moisture regime and nitrogen levels on soil CO₂ evolution (mg kg⁻¹)

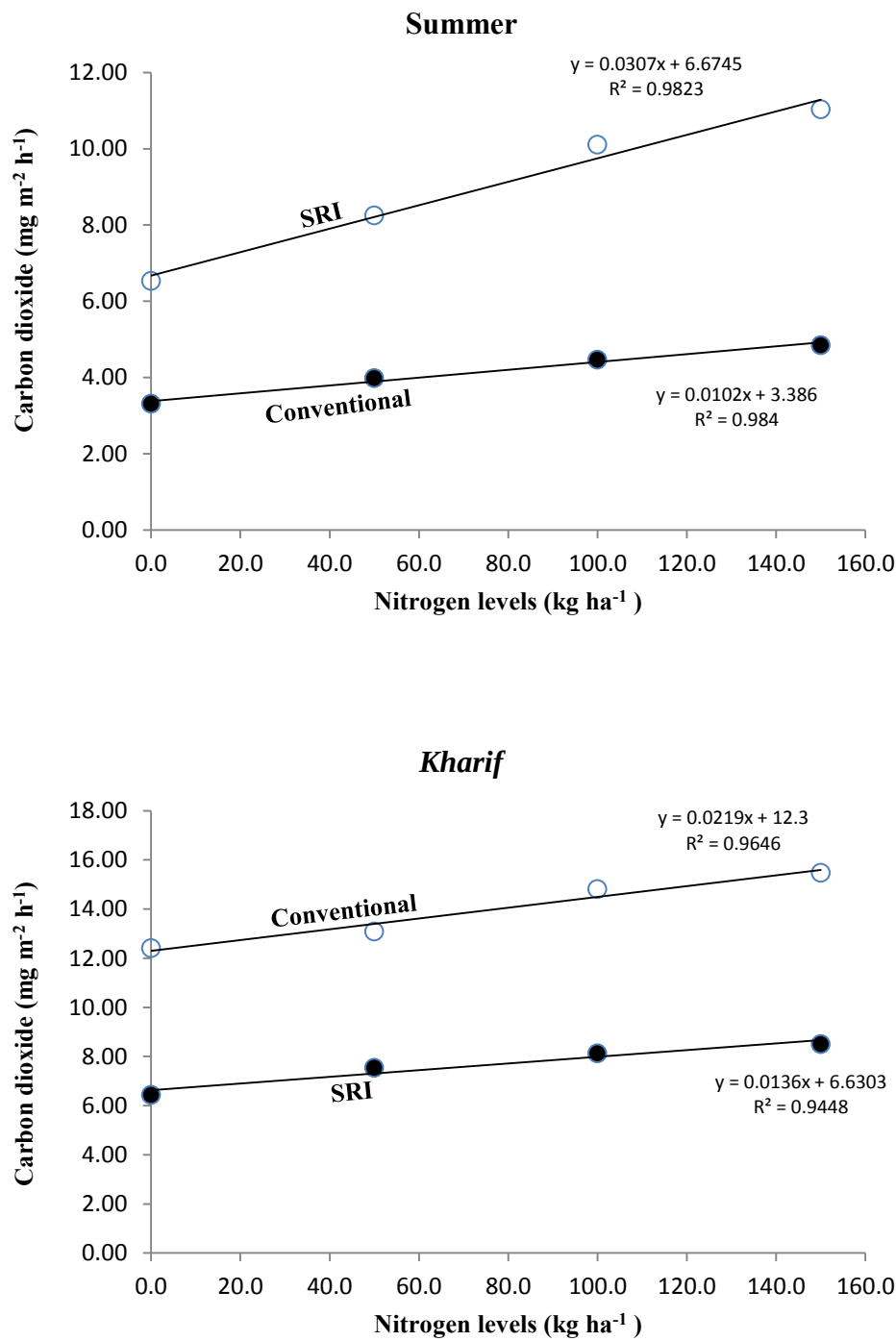


Fig 5.25. Correlation between carbon dioxide emission and nitrogen doses

5.4. Soil biochemical changes

A distinct difference in the soil biology of two systems of rice cultivation was observed. The SRI recorded consistently higher numbers of bacterial, fungal and actinomycetes populations than conventional system. This is evident from the fact that the SRI system which makes the soil more aerobic besides producing large root system that might have favoured for enhanced populations of beneficial soil microorganisms. Our observations are in conformity with the findings of several researchers (Mishra and Salokhe, 2008; Thakur *et al.*, 2010; Zhao *et al.*, 2010) who have reported that the SRI management could affect principal factors that apparently contribute for the abundance diversity of soil biota. This is also supported by Gyathry (2002) and Iswandi *et al.* (2011) who have reported that the SRI soils had increased the total bacterial populations by 312 per cent and 65 per cent over conventional systems, respectively. The observations of Iswandi *et al.* (2011) also add strength to our results who reported that the SRI system with large root systems producing more root exudates coupled with aerobic soils are the crucial factors for supporting diverse populations of beneficial (aerobic) soil biota. Nitrogen fertilization was found to enhance the soil microbial populations irrespective of the systems of rice cultivation. This is obvious for the reason that nitrogen is essential for the microbes as a source of nutrient besides it facilitates to maintain desirable C:N ratio of soils.

Soil enzymes namely dehydrogenase, phosphatase and urease were estimated. The data clearly indicated that the SRI system with intense microbial populations has directly contributed for enhanced soil enzyme activities. Dehydrogenase is used as an indicator of viable microbial activity (Alkorta *et al.*, 2003). Since it exists as an integral part of intact cells and this enzyme reflects the total oxidative activities of soil microflora, important in oxidizing soil organic matter (Dick, 1997). In the present investigation, the SRI system had increased the dehydrogenase activity by 8.4 to 11.4 per cent and 10.1 to 12.3 per cent, respectively during summer and *kharif* seasons. These results are in accordance with the observations of several workers (Gyathry, 2002; Rupela *et al.*, 2006; Iswandi *et al.*, 2011) who have reported that the SRI system increased the dehydrogenase activity to the tune of 140, 22.5 and 125 per cent over the conventional systems, respectively.

Phosphatase activity was also higher in SRI than conventional system and this might be due to the fact that the SRI soils are relatively aerobic and hence it would have favored the growth of phosphate-solubilizing bacteria (Uphoff *et al.*, 2009). This is also supported by ample literatures (Gyathry, 2002; Rupela *et al.*, 2006; Iswandi *et al.*, 2011) which reported that the SRI soils had increased the P solubilizing microbes by 53, 3.6 and 78 per cent over conventional systems, respectively. In addition, Gyathry (2002) reported that the SRI soils had consistently higher phosphatase activity in comparison to conventional system. Urease activity was higher in SRI soils than conventional system, however the values were non-significant. Urease belongs to a group of enzymes acting on the carbon-nitrogen (C-N) bonds of urea (Alkorta *et al.*, 2003) and hence nitrogen fertilization significantly increased these activity irrespective of the systems of rice cultivation. Similar findings were reported by Dick *et al.*, 1988 and McCarty *et al.*, 1992 who have recorded increased urease activity as a result of urea fertilization. In this study, the nitrogen fertilization, in general increased all the three soil enzyme activities. This is obvious that the nitrogen fertilization enhances diverse soil microbial populations and thereby increased the soil enzyme activities. The microbial population closely corroborates with the estimated soil enzyme activities.

5.5. Influence on above ground and below ground biomasses

This study brought out some interesting facts on the productivity of two distinct systems of rice cultivation. The biometric observations such as tillers per hill, productive tillers per hill, root biomass per hill and root volume revealed the superiority of rice plants when grown under the SRI system of rice cultivation in comparison to conventional system (Plate7). The profuse tillering in the SRI system is promoted by its sets of management practices, including the transplantation of young seedlings, square planting, cono-weeding operation and intermittent wetting and drying water management strategy. Among these practices, the transplantation of young seedlings is considered to an important factor in promoting profuse tillering. The benefits of transplanting rice seedlings at a younger stage have been confirmed by many researchers (Yamamoto *et al.*, 1995; Randriamiharisoa and Uphoff, 2002; Horie *et al.*, 2005). This practice capitalizes on the fact indicated in Katayama theory on tillering model that the young rice seedlings before start of four phyllochron (less than 15 days) have the potential to produce large number of

Plate 7 . Comparison of conventional and SRI rice hill at harvest



tillers and roots simultaneously (Katayama, 1951). Fr. de. Laulanie, the father of SRI also observed that if transplanting is done beyond the fourth phyllochron (after about 15 days), the first primary tiller does not emerge and all of the descendents of this tiller are lost (Thiyagarajan *et al.*, 2009). Further, the transplanting of younger seedlings is advantageous for early crop establishment besides avoiding/minimizing of transplanting shock (Pasuquin *et al.*, 2008). Therefore, the profuse tillering ability of rice plants under SRI system has a close relationship with the number of phyllochrons completed before entering the reproductive stage (Nemoto *et al.*, 1995; Stoop *et al.*, 2002).

In addition to the phyllochron theory on tillering potential, the other two factors that may play a vital role in producing large number of tillers in SRI are square planting and cono-weeding operation. Square spacing reduces inter-plant competition for nutrients, water, light, and air which accounts for a significant enhancement in individual hill performance under SRI (Thakur *et al.* 2010). The soil churning by cono-weeder has a specific positive response on the growth of plants and it significantly increased the tiller production (Randriamiharison, 2002; Thiyagarajan *et al.*, 2009).

In the present study, it was observed that the rice plants under the SRI system of rice cultivation produced larger root biomass per hill and root volume in comparison to conventional system. The increased root biomass per individual plant in SRI is as a result of the synergistic effects of transplanting young seedling, square planting, four times of cono-weeding and alternate wetting and drying. Our observations are supported by previous studies (Mishra and Salokhe, 2008) which reported that younger seedlings used with SRI, perform better in terms of various root characteristics (root length density and root weight density) than older seedlings.

One of the key factors in SRI that facilitates in improved root growth is the water management strategy that keeps the soil almost aerobic during the vegetative stage of crop growth (Bouman *et al.*, 2007). Aerobic soil conditions are generally more favorable for root functioning compared to anaerobic circumstances (Kirk and Bouldin, 1991; Kirk and Solivas 1997; Drew, 1997). It is well established by several researchers (Friend *et al.*, 1990; Sasaki and Hoshikawa, 1997) that oxygenated soil promotes better nodal root development (Mishra and Salokhe, 2008) by improving the root initiation and thereby

directly contributes for better root biomass. This might be due to the N status of the soil that governs the rooting pattern. A higher ratio of $\text{NH}_4^+ : \text{NO}_3^-$ favours greater cytokinin production that helps support the elongation of roots whereas a higher $\text{NO}_3^- : \text{NH}_4^+$ ratio favours the production of auxins, which favour root initiation (Debi *et al.*, 2005). That is why NO_3^- is considered to induce root development (Friend *et al.*, 1990). Our field data on N fractions also showed that SRI soils had relatively higher amounts of NO_3^- in comparison to the conventional system. These facts also help us to explain the reason for better root growth in SRI system.

Conversely, the continuous flooding in conventional system can have suppressive effects on rice root systems, most notably the deformation of their cortex and creation of aerenchyma (air pockets) (Kirk and Bouldin, 1991), with consequent degeneration of 78 per cent of the roots by flowering stage (Kar *et al.* 1974). Therefore, in the conventional system of rice cultivation, the hypoxic soil conditions caused by inundation would have limited the roots' ability to respire and ultimately resulted in the slowdown of root metabolism, ion transport and decreased root growth (Barison and Uphoff, 2011).

In addition to the above explained factors that were responsible for improved root biomass in SRI, the cono-weeding operation deserves a special mention. Four times of cono-weeding during the early stages of rice crop helps to prune some of the upper roots and thereby encourages deeper root growth (Uphoff, 2002). Overall, our observations on root biomass per hill and root volume are in conformity with the ample number of research publications (Rupela, *et al.*, 2006; Uphoff *et al.*, 2009; Barison and Uphoff, 2011).

Despite the fact that the rice plants in SRI were superior in terms of highest number of tillers, productive tillers and root biomass per hill, our observations on these parameters including the above ground and below ground biomasses per unit area basis revealed a different scenario. The conventional system recorded the higher number of productive tillers and root biomass per m^2 than SRI. This is evident from the fact that the decreased plant density (16 hills per m^2) in SRI was the reason for registering lower values when observed per m^2 basis. Similar observations were made by Thakur *et al.* (2009) who indicated that wider spacing (25 x 25 cm and 30 x 30 cm) in SRI produced the lowest number of productive tillers and root biomass per unit area basis despite the higher

number of tillers and root biomass per hill. Planting density in rice is known to play a crucial role for dry matter production and grain yield (Yoshida, 1981). At wider spacing, the dry weight of individual hills increased significantly compared with closely-spaced hills, irrespective of practices (Thakur *et al.*, 2009). Therefore, it is once again emphasized from our observations that crop yields are the result of performance of the total plant community rather than of individual hills.

There is an ongoing debate among the rice scientists across the globe on the yield potentials of SRI. Uphoff (2002) claimed that under SRI system rice yields may go up to 15 to 20 Mg ha⁻¹. Further, Stoop *et al.* (2002) reported that the synergies among these unconventional management practices unlock the physiological potential of rice, with results that challenge the prevailing notions of yield barriers in rice. However, these claims have been questioned (Dobermann, 2004; Sheehy *et al.*, 2004). Interestingly, our results from two field experiments revealed that the above ground biomass (grain yield + straw yield) and below ground biomass (root biomass) was significantly higher in conventional system than SRI. This suggests that the population reduction of one-third caused by the spatial geometry in SRI is unable to compensate to the level of conventional system (Fig.5.26). Despite the fact that number of productive tillers per hill of SRI was higher to the tune of 53 to 62 per cent, it was inadequate enough to match the loss on per hectare basis. This observation is very unique to bring out a critical attribute that contributes for the total crop productivity under conventional system in comparison to SRI. Our results are in accordance with the observations of several scientists (Sheehy *et al.*, 2004; Latif *et al.*, 2005; Thakur *et al.* 2009; Anitha and Chellappan, 2011) who reported lower yields in SRI. They concluded that the rice plants in SRI adapts well to decreased plant density by increasing yield per plant but this was apparently not enough to compensate for the reduction in plant density.

Based on our grain yield data with the spacing of 25 x 25 cm, we hypothetically predicted the SRI yield potential with different spacings *vis-à-vis* 22.5 x 22.5 cm and 20 x 20 cm and were compared with the yield obtained in conventional method (20 x 10 cm). From the calculations, it is concluded that when SRI is practiced with the spacing of 22.5 x 22.5 cm and 20 x 20 cm it can out yield conventional system (Table 5.1). Our hypothesis is further strengthened from the field results of Thakur *et al.* (2009) who

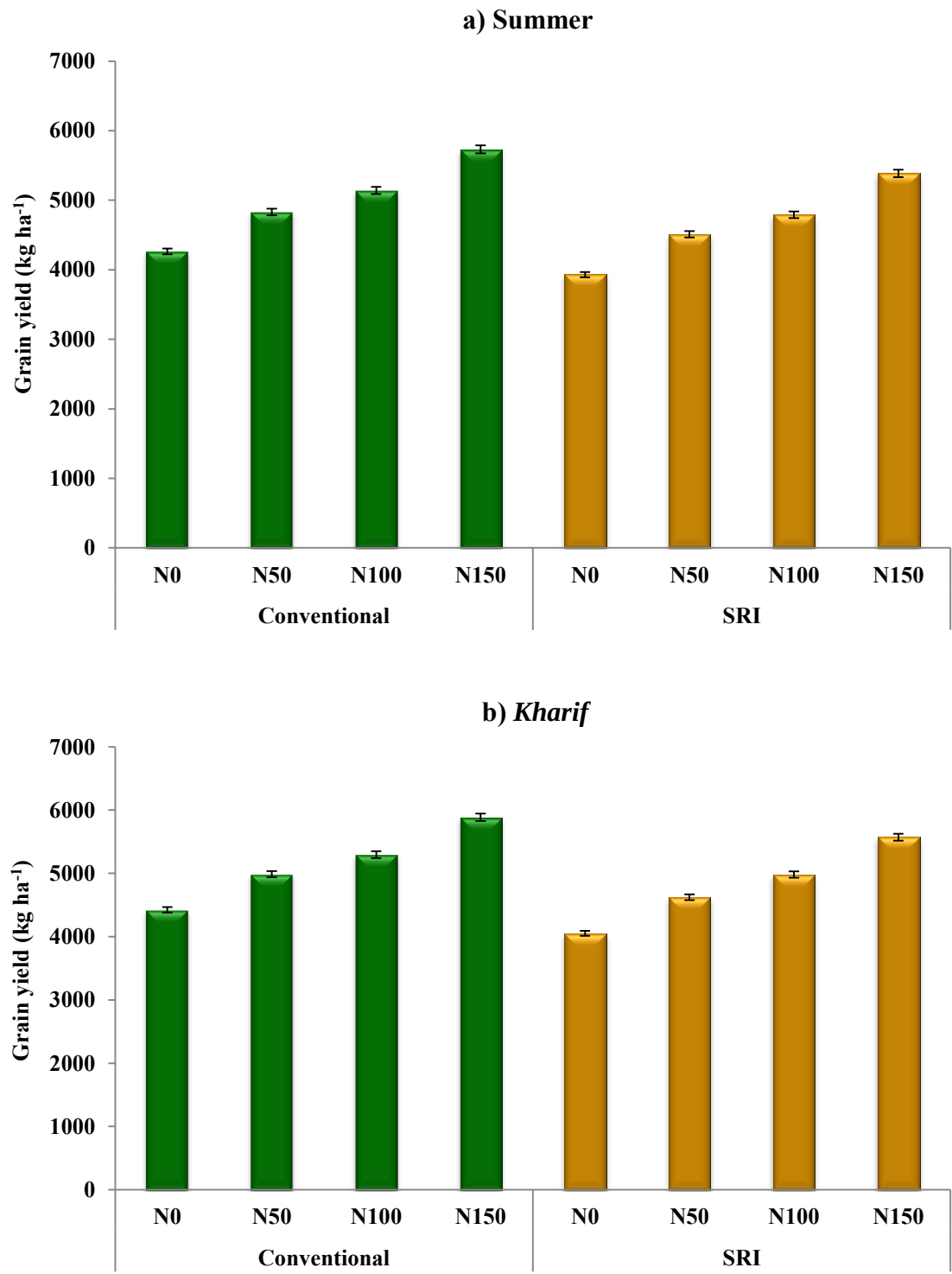


Fig. 5.26. Effect of systems of rice cultivation and different levels of nitrogen on rice grain yield

Table 5.1. Hypothetical Prediction of SRI Yield Potential

System of Rice cultivation	Plant Spacing	Population		Grain yield (kg ha ⁻¹)
		Plants per m ²	Plants per ha	
Conventional method	20 cm x 10 cm	50	5,00,000	5071*
SRI	25 cm x 25 cm	16	1,60,000	4731*
	22.5 cm x 22.5 cm	20	2,00,000	5913
	20 cm x 20 cm	25	2,50,000	7391

* mean of recorded yield in the present study

evaluated the performance of SRI with different plant spacings and reported that the SRI plants with the spacing of 20 x 20 cm out-yielded the conventional method whereas the spacing such as 25 x 25 cm and 30 x 30 cm registered lowest yields.

5.6. Effect of elevated [CO₂] on SOC dynamics

The rice plants were exposed to elevated carbon dioxide concentrations ([CO₂]) viz., 550 and 750 $\mu\text{mol mol}^{-1}$ and its effects on SOC dynamics were investigated. The results revealed that the elevated [CO₂] significantly increased the SMBC and DOC throughout the cropping period. Enhanced photosynthesis and plant growth under elevated [CO₂] had led to increased C input to the soil (including cortical cell sloughing, root exudation and mortality) (Ineson *et al.*, 1996; Cheng and Johnson, 1998). As soil microorganisms are often C limited (Anderson and Domsch, 1986; Smith and Paul, 1990), more C input will directly contribute for increased soil microbial biomass and activities. Moreover, in submerged rice soils enhanced algal growth in response to CO₂ enrichment was reported to increase microbial biomass of the surface soil (Inubushi *et al.*, 1999; Inubushi *et al.*, 2011).

Increasing atmospheric [CO₂] is unlikely to directly influence soil microorganisms because CO₂ concentrations in soils are already 10–50 times higher than in the atmosphere (Lamborg, 1983; Schortemeyer *et al.*, 1996), even though it usually stimulates plant (especially C₃ plant) productivity due to higher net carbon assimilation (Kimball *et al.*, 1993). Nevertheless, elevated atmospheric [CO₂] may indirectly affect soil microbial populations (Montealegre *et al.*, 2002), since root biomass, total rhizodeposition, and chemical composition of plant tissues and root exudates probably change when atmospheric CO₂ is enriched (Rogers *et al.*, 1994; Schortemeyer *et al.*, 1996). Our results are in conformity with Li *et al.* (2004) who reported that SMBC, SMBN, DOC had positive relationships with elevated [CO₂]. In the present investigation, the SMBN (soil microbial biomass nitrogen) was also enhanced under elevated [CO₂] which is in line with the reports (Diaz *et al.*, 1993; Zak *et al.*, 1993; Niklaus, 1998; Li *et al.*, 2004). Nitrogen fertilization significantly increased SMBC, SMBN and DOC irrespective of the [CO₂]. This is attributed to the fact that addition of nitrogen favours increased plant biomass and in turn it contributes for more root exudates which serves as a source of energy for soil microbial population. On the other hand, low N supplement limited the enhancement of root growth

by elevated $[\text{CO}_2]$, leading consequently to diminished response of SMBC, SMBN and DOC to CO_2 enrichment (Li *et al.*, 2004). According to Cardon (2001), the influence of elevated CO_2 on soil microbial population is linked to the nutrient status of the soil. Under nitrogen limited conditions, effects of elevated CO_2 on plants were generally found to be much smaller (Korner *et al.*, 1997) besides it is also suggested that poor N supply limited the microbial utilization of C compounds (van Veen *et al.*, 1991).

Though significant changes in the active pools of soil carbon were observed under elevated $[\text{CO}_2]$, the measurements on SOC revealed no alterations during the experimentation. The data are in agreement with the results of Cheng *et al.* (2001) who have reported that the elevated $[\text{CO}_2]$ did not alter the SOC despite plant-derived C (rhizo-deposition) was greatly enhanced but disappeared due to rapid decomposability. Most field scale CO_2 enrichment studies have failed to detect significant changes in SOC leading to the general conclusion that the potential for increased soil C is limited (Hungate *et al.*, 1997; Gill *et al.*, 2002; Hagedorn *et al.*, 2003; Lichter *et al.*, 2005). Yet, it is currently unclear whether the lack of change in these studies is a general response or a function of (1) the low statistical power of most experiments and/or (2) the magnitude of CO_2 stimulated C inputs relative to the duration of the experiments (Filion *et al.*, 2000; Smith, 2004; Hungate *et al.*, 2009). Nitrogen fertilization significantly decreased SOC at all the levels of $[\text{CO}_2]$. This is in line with Sillen and Dieleman (2012) who reported that nitrogen additions promoted decomposition processes also under elevated $[\text{CO}_2]$ and thereby reducing the potential for increased soil C storage.

5.7. Effect of elevated $[\text{CO}_2]$ on methane emissions

The data clearly indicated that methane emissions were significantly higher under $[\text{CO}_2]$ of $750 \mu\text{mol mol}^{-1}$ by 33 to 54 per cent over the ambient $[\text{CO}_2]$ of $380 \mu\text{mol mol}^{-1}$ (Fig.5.27). Our results are in line with ample number of literatures which reported that elevated $[\text{CO}_2]$ enhanced the methane emissions by 49 to 60 per cent @ $650 \mu\text{mol mol}^{-1}$ (Ziska *et al.*, 1998), 38 to 51 per cent @ $550 \mu\text{mol mol}^{-1}$ (Inubushi *et al.*, 2003), 58 per cent @ $700 \mu\text{mol mol}^{-1}$ (Cheng *et al.*, 2006) and 26 per cent @ $580 \mu\text{mol mol}^{-1}$ (Tokida *et al.*, 2010) over the ambient $[\text{CO}_2]$. These facts are further supported by a recent meta-analysis report (van Groenigen *et al.*, 2011) which indicated that $[\text{CO}_2]$ between 463 to $780 \mu\text{mol mol}^{-1}$

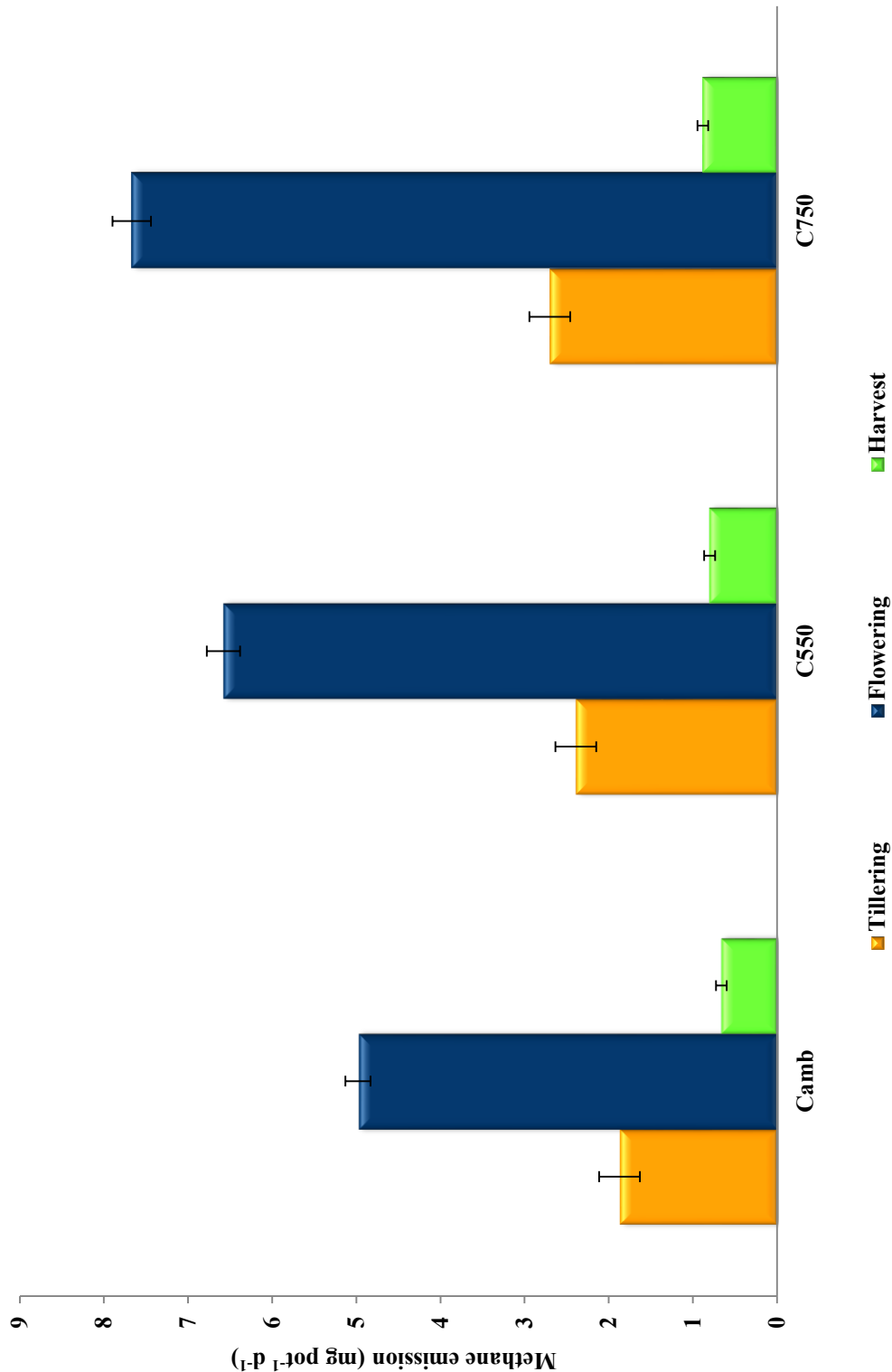


Fig.5.27. Effect of elevated [CO₂] on methane emission at rice growth stage

stimulated CH₄ emissions on an average by 43.4 per cent. CH₄ is the dominant terminal degradation product of soil organic materials in submerged rice fields (Kruger *et al.*, 2001), therefore, increased C input to the soil in response to elevated [CO₂] leads likely to enhanced CH₄ production (Ziska *et al.*, 1998; Li *et al.*, 2004). Moreover, the positive correlations between CH₄ emissions and above-ground or root biomass also agree well with the results of previous studies suggesting that greater assimilation of carbon under high [CO₂] leads to higher rates of rhizo-deposition (root exudation and autolysis products), which is an important source of substrates for CH₄ production (Inubushi *et al.*, 2003; Xu *et al.*, 2004; Tokida *et al.*, 2010). Further the positive correlation of DOC with methane emissions also adds strength to our results. Among the different stages of observation, the flowering stage recorded the highest methane flux irrespective of [CO₂]. This period generally correspond with increased availability of root sloughing or exudates as a result of peak photosynthetic activity as well as advanced root senescence and might probably provided more substrate for methanogenesis (Allen *et al.*, 2003; Tokida *et al.*, 2010). The results from this experiment also demonstrated that nitrogen fertilization increased the methane flux irrespective of [CO₂]. Inferring from these observations, it is reasonable to conclude that the application of N fertilizers enhances rice biomass and contributed for enhanced root exudates that favours methane production (Schimel, 2000).

In order to understand the mechanisms associated with methane flux under elevated [CO₂], the population dynamics of methanogens and methanotrophs were studied. Consistent with the observed increases in methane flux, the enumeration of methanogens showed a significant increase under elevated [CO₂]. This positive effect of elevated [CO₂] may be attributed to stimulated rice above ground and below ground biomasses (Ziska *et al.*, 1998) which might have provided more carbon substrates for methanogens (Hou *et al.*, 2000; Inubushi *et al.*, 2003; Yue *et al.*, 2003; Yue *et al.*, 2007). Root exudation accounts for approximately 0.5–5.0 per cent of net fixed C (Farrar and Jones, 2003) and provides 10 and 50 per cent of the carbon substrate needed for methanogenesis (Seiler *et al.*, 1984). In addition, the cells are sloughed from the cortices of living roots and lysates consisting of polymeric C compounds and enzymes enter the rhizosphere, providing further substrate for microorganisms (Farrar *et al.*, 2003). This fact also supports our observations on methanotrophs, which was found to be higher under

elevated [CO₂]. Interestingly, the data showed that the highest methanotrophic population was found at the harvest stage and these observations are in accordance with (Yue *et al.*, 2007) who reported enhanced populations of methanotrophs at maturing periods under elevated [CO₂]. This suggests that the availability of O₂ and the concentration of CH₄ jointly determine the methanotrophic activity (van Bodegom *et al.*, 2001).

5.8. Effect of elevated [CO₂] on rice physiology

The physiological parameters such as chlorophyll contents, soluble protein, nitrogen assimilatory enzymes and anti-oxidant enzymes were estimated to gain insights on the response of rice plants under elevated [CO₂] with varying levels of nitrogen. In the present study, the chlorophyll a, b and total chlorophyll contents were significantly higher under elevated [CO₂] at the tillering stage, but the trend was reversed with the advancement of crop growth (Fig.5.28). The increased chlorophyll contents at the tillering stage under elevated [CO₂] is due to the fact that the short-term CO₂ enrichment stimulates the rate of photosynthesis (Makino and Mae, 1999) and simultaneously increases the nitrogen uptake (Seneweera *et al.*, 2005). This statement is also supported by our results on leaf nitrogen content (Fig.5.29) which had positive correlation with chlorophyll contents. On the other hand, the decline in leaf chlorophyll in response to CO₂ enrichment cannot be solely explained by a dilution effect (Epron *et al.*, 1996). Such a decrease in leaf chlorophyll has frequently been reported in young plants in response to elevated CO₂ (Delucia *et al.*, 1985, Houppis *et al.*, 1988, Wullschleger *et al.*, 1992) and may have some physiological significance. Decrease in chlorophyll content at the later stages under atmospheric CO₂ enrichment suggests that elevated [CO₂] causes less nitrogen to be invested in the light reactions of photosynthesis (Epron *et al.*, 1996). Further, many studies suggest that decreases in leaf nitrogen and chlorophyll content under elevated CO₂ levels are often associated with reduced responses of leaf photosynthesis (Nakano *et al.*, 1997; Kim and You, 2010). In general, a decreasing trend in the chlorophyll contents was observed with the progression of crop growth irrespective of [CO₂]. This observation is line with Imai *et al.*, (2005 and 2008) who reported that the chlorophyll contents increased in young expanding leaves and decreased substantially during senescence. Nitrogen fertilization was found to increase the chlorophyll contents irrespective of [CO₂] or stages of observation. In any species, a large proportion of N is allocated to leaves throughout their life cycle. For e.g., in rice, at

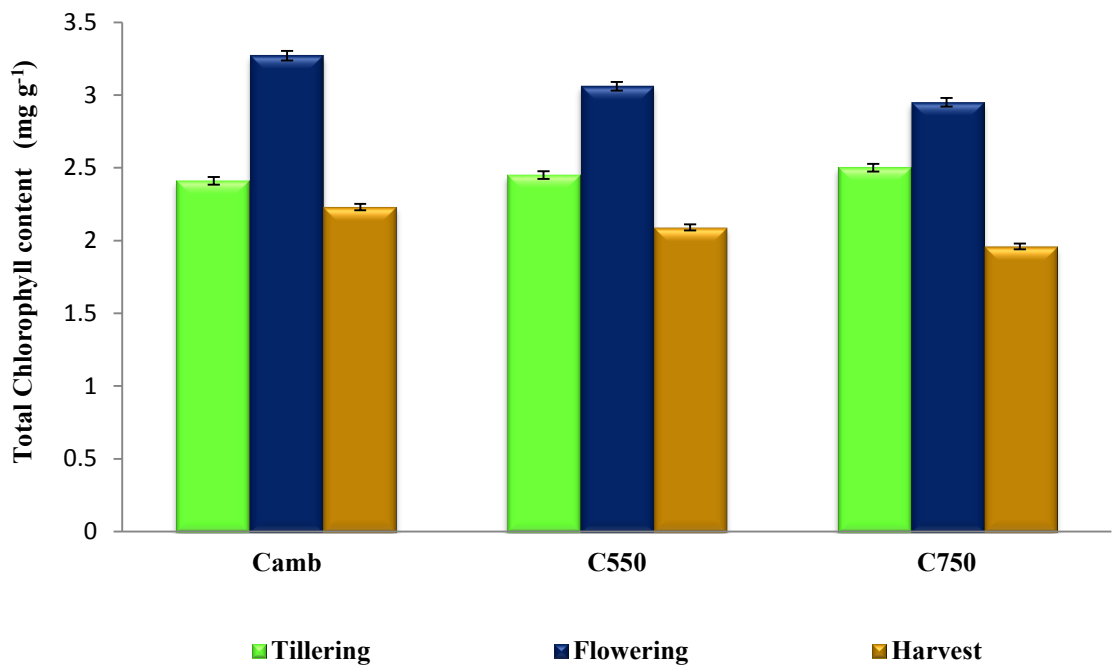


Fig.5.28. Effect of elevated [CO₂] on total chlorophyll content at various stages of rice growth

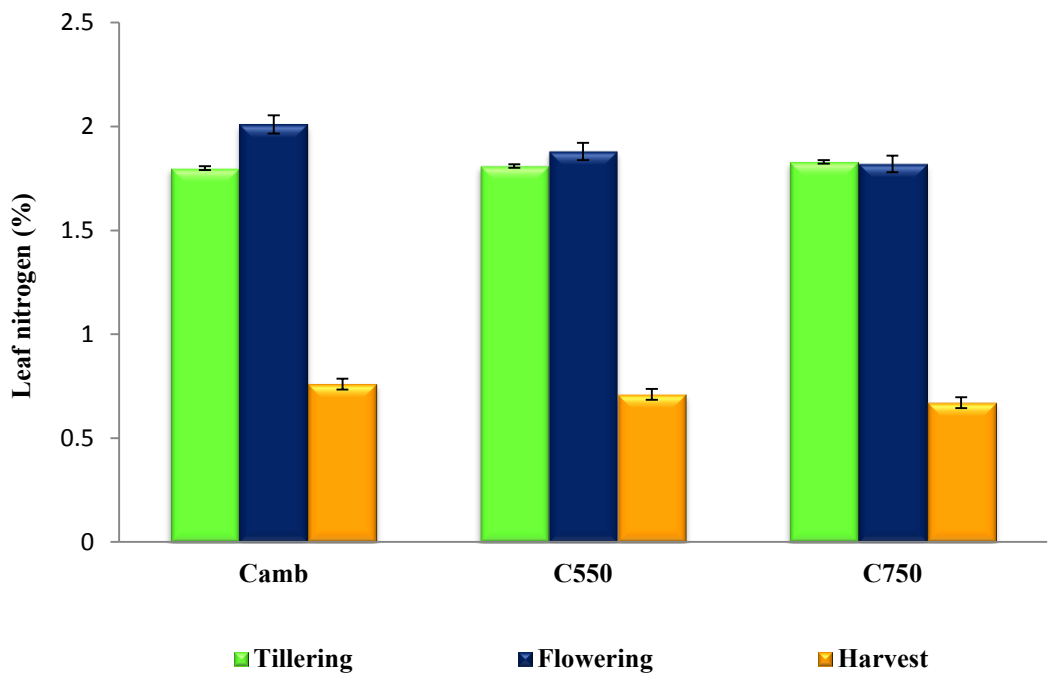


Fig.5.29. Effect of elevated [CO₂] on leaf nitrogen at various stages of rice growth

early panicle initiation stage about 65-70 per cent of the total N in the shoot is invested in the leaf blade and 20 to 30 per cent in the leaf sheath (Seneweera *et al.*, 2005). A major proportion of leaf N (~80 per cent) is allocated to chloroplasts and most of the N in the chloroplast is invested in photosynthetic protein, including large amounts in Rubisco and thylakoid protein (Evans, 1989).

In this investigation, the soluble proteins were estimated as it accounts for more than 50 per cent of rubisco content (Mae *et al.*, 1983; Suzuki *et al.*, 2001) besides it is the reliable index for assessing the photosynthetic efficiency (Jensen and Bahr, 1977). The soluble proteins increased in the rice plants exposed to elevated [CO₂] at the tillering stage, but the trend of response was reversed with the advancement of crop growth. Since soluble proteins are an indirect measure of rubisco content, this can be well explained with the literatures dealing rubisco content under elevated [CO₂]. It has been demonstrated that under elevated [CO₂], rubisco content in the leaf is varied during development (Seneweera *et al.*, 2002) and it was found to be significantly reduced at the reproductive stage. These changes in rubisco content are related to modulation in C and N balance due to changes in source and sink activity during development but the underlying mechanism is not clearly understood (Seneweera *et al.*, 2005). Another possibility is that plants reduced their N allocation for photosynthetic proteins, making nitrogen more freely available for rapid sink development. In the present study, even though the soluble proteins, an indirect measure of rubisco was found to be reduced at later stages under elevated [CO₂], the biomass harvested was significantly higher under the elevated [CO₂]. This is attributed to the fact that at elevated [CO₂], photosynthesis is not limited by rubisco content whereas electron transport capacity (Farquhar *et al.*, 1980) or availability of P_i in the chloroplast to synthesize ATP limits the photosynthesis (Sharkey, 1985). This data suggest that rubisco content is not necessarily important to maintain high photosynthetic rates at elevated [CO₂] and other regulatory mechanisms within the photosynthetic machinery may exist for downregulation of photosynthesis. Moreover, several reports indicate that the decrease in rubisco content is not accompanied by a decline in CO₂ assimilation rates because the activation state of rubisco increased (Rowland-Bamford *et al.*, 1991; Allen *et al.*, 2000; Cheng and Fuchigami, 2000).

Our study indicated that on an average the leaf nitrogen was significantly reduced by 10.6 per cent and 6.5 per cent during the later stages in the rice plants exposed to $[\text{CO}_2]$ @ $750 \mu\text{mol mol}^{-1}$ and $550 \mu\text{mol mol}^{-1}$, respectively over the ambient $[\text{CO}_2]$. These results are in agreement with Anten *et al.* (2003) who reported that the rice grown at elevated CO_2 ($200 \mu\text{mol mol}^{-1}$ above ambient) registered reduced leaf N by per cent. This decrease was found only after panicle initiation and the reason for these differences could be due to an increase in N demand for larger panicles in plants grown at elevated $[\text{CO}_2]$ (Seneweera *et al.*, 2002). One of the common explanations for the reduction in leaf N concentration in plants grown at elevated $[\text{CO}_2]$ is the dilution of N due to extra carbohydrate accumulation (Conroy, 1992). But several publications (Makino *et al.*, 1997; Nakano *et al.* (1997); Makino and Mae, 1999) disagrees the ‘dilution effect’ concept and instead it is explained that the decrease in leaf N content is the result of a change in N allocation of the whole plant. According to Makino and Mae (1999), the N investment in the leaf blades decreases under CO_2 enrichment irrespective of N treatments, whereas the plants enhance the allocation of N to the leaf sheaths and roots. This means that during long-term growth under conditions of elevated $[\text{CO}_2]$, plants reallocate N away from leaf blades to leaf sheaths and roots. In addition, since N invested into leaf blades is the most important source for photosynthesis in the whole plant, these results also indicate that plants regulate photosynthesis by changing N allocation at the whole plant level.

To further understand the underlying reasons for reduced plant tissue nitrogen under elevated $[\text{CO}_2]$, the nitrogen assimilatory enzymes viz., nitrate reductase (NRase), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH) in the rice leaves and roots were estimated. The ability of plants to acquire and assimilate N is an important determinant of plant response to elevated CO_2 and of ecosystem carbon sequestration (Stitt and Krapp, 1999; Luo *et al.*, 2004). In this study, the plants exposed to elevated $[\text{CO}_2]$ did not exhibit any variations in the NRase activity in leaves in comparison to ambient $[\text{CO}_2]$ at tillering stage. Interestingly, our data revealed that the NRase activity in leaves was decreased at flowering stage, whereas the NRase activity in roots was increased at the same stage. This suggests that despite increased N uptake under elevated $[\text{CO}_2]$, the reallocation of N away from leaf blades might have led to decreased NRase activity in leaves and increased NRase activity in

roots at later stages of crop growth. Plant N status is a function of both soil N availability and plant N uptake and assimilation capacity. As a rate-limiting step in nitrate assimilation, the reduction of nitrate is an important component of plant physiological response to elevated CO₂ and terrestrial carbon sequestration (Natali *et al.*, 2009). Both CO₂ and N enrichment had species specific impacts on NRase activity. Both above and belowground assimilation processes may be altered by increasing concentrations of atmospheric CO₂. Our results are in accordance with a large number of studies which reported a decrease in foliar NRase activity with CO₂ enrichment (Bloom *et al.*, 2002; Searles and Bloom, 2003). The other possible reason for the reduction in NRase activity in leaves is that foliar NO₃⁻ reduction may compete for reductant with Calvin cycle reactions when carbon assimilation is increased under elevated CO₂ (Bloom *et al.* 2002). Because of the potential competitive effect between NO₃⁻ and CO₂ reduction, foliar NRase may decrease with CO₂ enrichment. However, variations in response are widely reported and this may be attributed to soil N form and concentration (Kruse *et al.*, 2003; Yong *et al.*, 2007), plant species/functional group (Cousins and Bloom, 2003) and altered diurnal rhythm of NRase activity (Geiger *et al.*, 1998).

Carbon metabolism is inextricably linked to nitrogen metabolism and any change in carbon abundance impacts on nitrogen metabolism and vice versa (Noctor and Foyer, 2000; Lewis *et al.*, 2000). There is clear evidence that more than 95 per cent of the ammonium available to higher plants is assimilated via the GS/GOGAT pathway (Lea and Mifflin, 1974). In the present study, the trend of response for GS, GOGAT in rice leaves and roots were similar to the NRase activity. The GS and GOGAT activities at the flowering stage were higher in roots of elevated CO₂ which is closely coincided with N uptake besides enhanced photosynthetic activities which contributed for the ATP requirement of the GS-GOGAT cycle at the interface of N and C metabolism (Moorhead and Smith, 2003). On the contrary, GS and GOGAT activities of leaves have shown to be lower under elevated CO₂ indicating the process of remobilization of metabolites towards reproductive organs which in turn support grain growth. Moreover, the glutamate is the precursor for chlorophyll synthesis in leaves (Yaronskaya *et al.*, 2006) and therefore the positive correlation of chlorophyll contents in leaves with GS and GOGAT activities can be taken as a support for our interpretation.

Rice plants exposed to elevated CO₂ levels had an inhibitory effect on GDH activities in both roots and leaves. Since, the physiological role of plant GDH has been a controversial issue for several decades (Miyashita and Good, 2008), it is hard to interpret our observation with limited dataset. However, the decreased GDH activities under elevated [CO₂] may be attributed to the fact that GDH plays a central role in amino acid breakdown under C deficient conditions and therefore enhanced carbon accumulation in rice plants under higher [CO₂] might decreased these activities. Several publications (Lea and Miflin, 1974; Robinson *et al.*, 1991; Miyashita and Good, 2008) have reported that GDH usually do not function in the normal metabolic assimilation of ammonia but rather in glutamate catabolism, providing carbon skeletons for the tricarboxylic acid cycle under C limited conditions and thus, acting as a link between carbon and nitrogen metabolism. However, the effect of elevated [CO₂] is still unclear considering the inconclusive scientific database on GDH activities.

In light of the important role of antioxidants in scavenging reactive oxygen species (ROS) and the inevitability of rising global atmospheric [CO₂], the effects of CO₂ enrichment on anti-oxidant enzyme activities were studied. The results clearly indicated that the anti-oxidant enzymes activities *viz.*, catalase, SOD (superoxide dismutase), peroxidase were decreased in the rice plants exposed to elevated [CO₂]. Similar reductions in anti-oxidant enzymes activities have been reported in the soybean (Pritchard *et al.*, 2000). Rice plants in CO₂ enriched environments typically enhances growth and photosynthesis by directly increasing the amount of carbon available for fixation, decreasing CO₂ lost to photorespiration and reducing oxygenase activity of rubisco (Lawlor and Mitchell, 1991). Several mechanisms have been advanced to explain how growth in elevated [CO₂], may reduce the amount of ROS formed within plant cells, thereby leading to reductions in constitutive antioxidant activity. First, increased CO₂/O₂ ratios within chloroplasts may decrease electron leakage from photosystem I to O₂, thereby attenuating O₂ formation (Schwanz *et al.*, 1996b). Second, increased ratios of CO₂/O₂ would decrease oxygenase activity of rubisco in favor of carboxylation, thus reducing photorespiration and resultant cellular H₂O₂ production. Third, because antioxidant activities are stimulated by water stress (Smirnoff, 1993; Polle, 1996; Schwanz *et al.*, 1996a) and growth in elevated [CO₂]

is often reported to alleviate water stress (Rogers *et al.*, 1983), there is a possibility of decreased antioxidant activities. Regardless of the mechanisms involved, it appears that constitutive antioxidant activity was down-regulated by growth in elevated CO₂.

5.9. Influence of elevated [CO₂] on biomass and carbon stock

The carbon sequestration potential in terms of above ground and below ground biomasses of rice crops exposed to elevated [CO₂] at varying levels of nitrogen were assessed. The data demonstrated that elevated [CO₂] @ 750 $\mu\text{mol mol}^{-1}$ significantly increased the above ground biomass (straw and grain) by 15.6 and 40.1 per cent, respectively, over the ambient [CO₂] of 380 $\mu\text{mol mol}^{-1}$. Interestingly, the rice plants under elevated [CO₂] of 750 $\mu\text{mol mol}^{-1}$ largely increased their below ground biomass (root) by 54.8 per cent over the ambient [CO₂]. In the case of rice plants exposed to elevated [CO₂] @ 550 $\mu\text{mol mol}^{-1}$, the above ground (straw and grain) and below ground biomass (root) were significantly increased to the tune of 9.3 and 24.0, 34.2 per cent, respectively over the ambient [CO₂]. These results are in agreement with previous experiments (Kim *et al.* 2003; Baker 2004; De Costa *et al.*, 2006; Yang *et al.*, 2006a; Krishnan *et al.*, 2007; Madan *et al.*, 2012) and modeling studies (Bannayan *et al.*, 2005) that reported enhanced rice biomass as a result of CO₂ enrichment. The increase in aboveground biomass with elevated [CO₂] is mainly attributed to increased photosynthesis and tiller number (Baker *et al.*, 1996; Sakai *et al.*, 2001; Ziska *et al.*, 1997). This statement is further strengthened by our data on number of tillers which indicated higher values under elevated [CO₂]. Growth during the seedling stage is 'source-limited' and the carbohydrates increased by CO₂ enrichment are efficiently utilized for additional sink such as the development of new tillers or secondary shoots (Makino *et al.*, 1997). Further, the increased below ground biomass is as a result of increased C allocation to the belowground, changes in root morphology (Berntson *et al.*, 1998; Kim *et al.*, 2001; Ziska *et al.*, 1997). In this study, the root/shoot ratio of rice plants under elevated [CO₂] of (750 and 550 $\mu\text{mol mol}^{-1}$) was increased to the tune of 30.9 and 21.3 per cent, respectively over the ambient [CO₂]. This data is in agreement with Upreti *et al.* (2000) and Gai-ping *et al.* (2006) who reported that rice root/shoot ratio was significantly increased by 155 and 35 per cent under CO₂ enrichment in comparison to ambient

treatment. The increase in root/shoot ratio might be due to redistribution of carbon in plant tissues since more carbon can be allocated to the roots under elevated CO₂ concentrations (Gorissen and Cotrufo, 2000).

In the present investigation, the nitrogen fertilization was found to a crucial factor for tapping the carbon sequestration potential of rice plants under higher levels of [CO₂]. Ample number of researchers (Ziska *et al.*, 1996; Li *et al.*, 2004; Seneweera *et al.*, 2005; Kim *et al.*, 2011) unequivocally demonstrated that the highest nitrogen doses are pivotal for rice plants to harness the potentials of 'CO₂ fertilization effect'. So far the interactive effect of carbon and nitrogen on growth has mainly been attributed to two mechanisms. First, inhibition of leaf photosynthesis by carbohydrate accumulation after prolonged exposure to elevated [CO₂] tends to be stronger under low than under high N availability (Rogers *et al.*, 1996). Second, N uptake increases under elevated [CO₂] only when N availability is high (Stitt and Krapp, 1999). Numerous experiments have demonstrated that elevated [CO₂] greatly increased dry matter accumulation of rice, with the magnitude of the response depending on nitrogen level (Ziska *et al.*, 1997; Nakagawa and Horie, 2000; Shimono and Bunce, 2009).

Our observations revealed that the elevated levels of CO₂ not only enhanced the total biomass of the rice plants but also increased the carbon content of rice roots and straw. However, the carbon content in grains was unaltered. Rice plants exposed to elevated [CO₂] of 750 and 550 $\mu\text{mol mol}^{-1}$ registered an increase in the carbon contents of roots (8.5 and 2.6 per cent) and straw (16.5 and 12.2 per cent) over the ambient [CO₂]. These results are in conformity with Uprety *et al.* (2000) who reported 10 to 15 per cent increase in the carbon content of rice leaves, stem and roots exposed to elevated [CO₂] of 575 to 620 $\mu\text{mol mol}^{-1}$ over the ambient [CO₂] of 370 $\mu\text{mol mol}^{-1}$. Thus, the combined effect of increased total biomass and carbon content under elevated levels of CO₂ contributed for enhanced plant biomass carbon stock to the tune of 22.5 – 34.4 per cent, 23.5 – 45.9 per cent and 24.5-39.8 per cent, respectively through straw, roots and grains over the ambient [CO₂] (Fig.5.30). On the other hand, elevated [CO₂] significantly reduced the nitrogen content of rice straw, roots and grains by 5.6 -10.1 per cent, 6.9 – 12.1 per cent and 5.2 – 5.9 per cent, respectively over the current CO₂ concentrations (Fig.5.31). Our results are in accordance with several publications (Anten *et al.*, 2003;

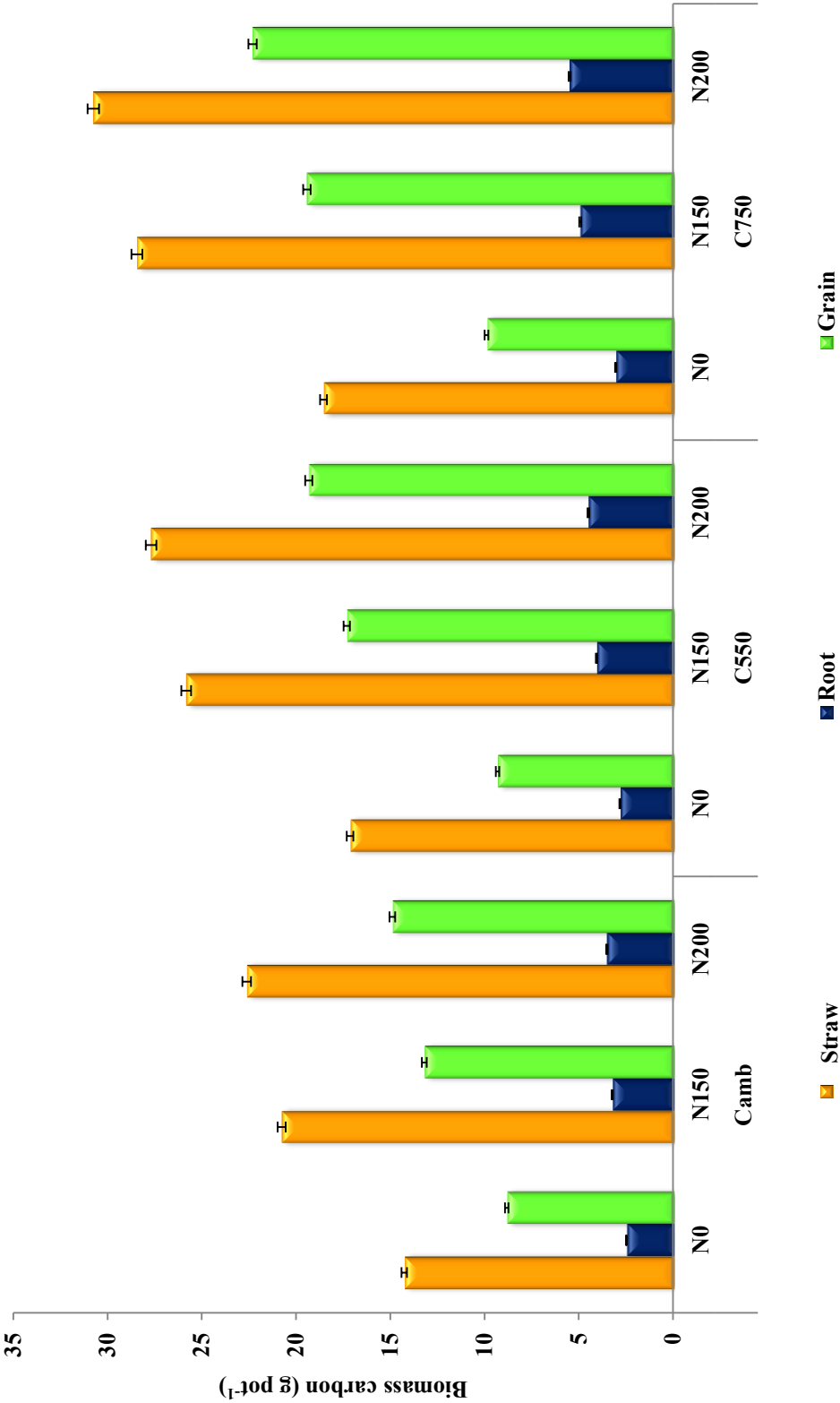


Fig.5.30. Effect of elevated [CO₂] and different levels of nitrogen on rice biomass carbon

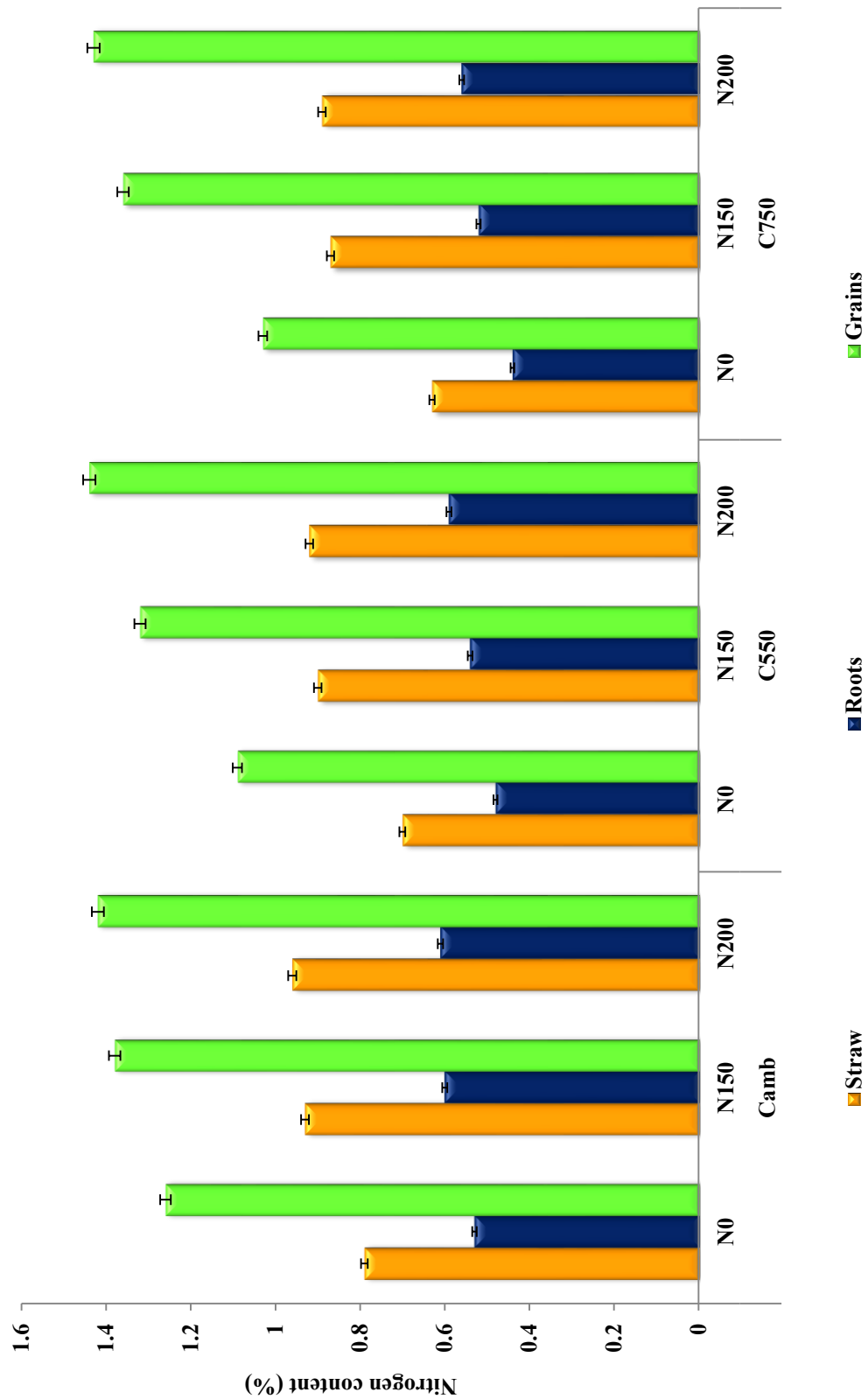


Fig.5.31. Effect of elevated [CO₂] on nitrogen content of rice straw, root and grain

Weerakoon *et al.*, 2005; Seneweera *et al.*, 2005). This is further supported by a recent literature (Kim *et al.*, 2011) who reported that the elevated $[\text{CO}_2]$ of $622 \mu\text{mol mol}^{-1}$ reduced the nitrogen content of rice shoot, roots and grains by 15.8 per cent, 16.9 per cent and 21.0 per cent, respectively over the current CO_2 concentrations of $380 \mu\text{mol mol}^{-1}$. It is obvious that these changes in carbon and nitrogen contents led to an increase in the C:N ratio of rice plants grown under elevated $[\text{CO}_2]$. From our results, it is observed that under elevated $[\text{CO}_2]$ the C:N ratio of rice roots, straw and grains were 82:1, 51:1 and 33.7 whereas the plants under ambient $[\text{CO}_2]$ recorded the C:N ratio of 66:1, 39:1, and 31:7 respectively. These observations are in conformity with large number of literatures (Upreti *et al.*, 2000; Anten *et al.*, 2003; Weerakoon *et al.*, 2005; Seneweera *et al.*, 2005; Gai-ping *et al.*, 2006) which reported that the CO_2 enrichment increased rice plant's C:N ratio.

5.10. Effect of elevated $[\text{CO}_2]$ on grain yield and quality

Environment cannot be protected unless man's basic needs are fulfilled. Hence, grain yield also comes into the domain of environmental scientists considering the saying 'poverty is the greatest polluter'. In this experiment, the results demonstrated that elevated $[\text{CO}_2]$ significantly increased the rice grain yield by 24.0 – 40.1 per cent over the current atmospheric $[\text{CO}_2]$ (Fig.5.32). This is in line with several findings (Kimball *et al.*, 2002; Baker, 2004; Kim *et al.*, 2011) which reported positive yield responses (5 to 71 per cent) to elevated CO_2 depending upon other factors such as temperature, cultivar and nutrition. Increased grain yield is evident from the fact that elevated CO_2 enhanced the accumulation of assimilation and the translocation from source (leaf or stem) to sink (panicle) (Sasaki *et al.*, 2005; Fan *et al.*, 2010). Among the yield components, panicle numbers, filled grains per panicle are the major contributors to the response of grain yield to elevated $[\text{CO}_2]$ (Imai *et al.*, 1995; Kim *et al.*, 2003; Yang *et al.*, 2006b). According to Imai *et al.* (1995) the grain number per panicle increased because high CO_2 overcomes the factors that cause flower degeneration during development. This might be the reason for registering highest grain number per panicle under elevated $[\text{CO}_2]$ in this study. In addition, the maximum temperature during the flowering period was less than 34.9°C irrespective of CO_2 levels, which might also helped to prevent the warming induced floral sterility in rice plants.

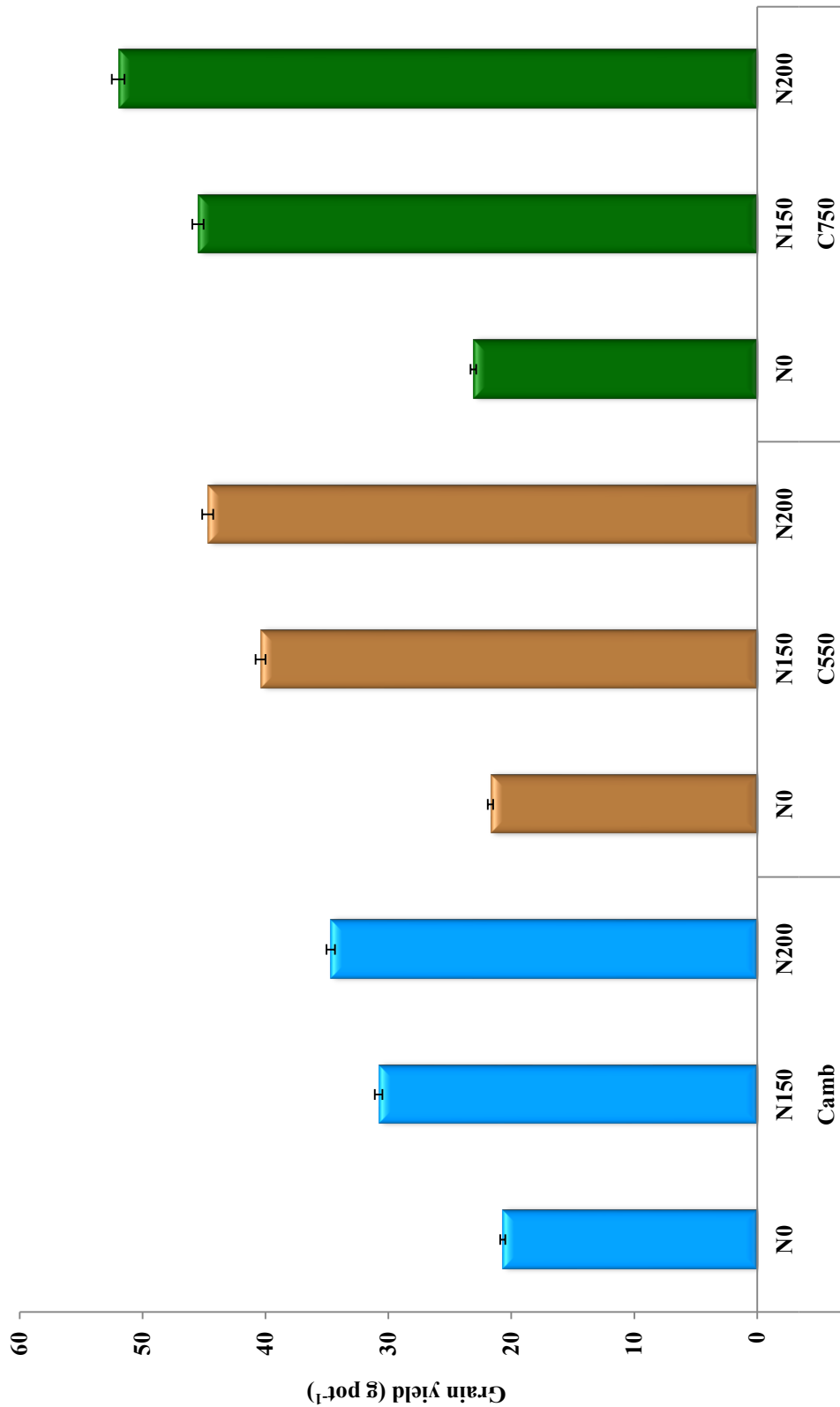


Fig.5.32. Effect of elevated $[CO_2]$ and different levels of nitrogen on rice grain yield

This study clearly demonstrated that the nitrogen fertilization is a crucial factor for preventing down regulation of photosynthesis and stimulating the grain yield under elevated CO₂ levels. Meta-analysis study (Ainsworth, 2008) pointed out that low N treatments negated any enhancement in rice yield at elevated [CO₂]. Low N fertilization limited N uptake during vegetative growth, which constrained any increase in spikelet number, thereby limiting the yield response (Kim *et al.*, 2003). Further, low N may also cause more pronounced acclimation of photosynthesis to elevated [CO₂], which can limit total dry matter (Suter *et al.*, 2001; Ainsworth *et al.*, 2003; Razzaque *et al.*, 2011).

Our results unequivocally demonstrated that nitrogen fertilization beyond the current recommended dose is pivotal under rising CO₂ concentrations for managing the grain quality in terms of nitrogen concentration. This observation is supported by several scientists (Perez *et al.*, 1996; Sasaki *et al.*, 2005; Kim *et al.*, 2011; Wang *et al.*, 2011) who reported that elevated [CO₂] significantly decreased nitrogen or protein concentration in rice grains. This suggests that when adequate N was not supplied, rice plants grown at high CO₂ became inferior to plants grown at ambient CO₂ in terms of grain quality. Though our results reported increased N uptake under elevated [CO₂], grain N concentration decreased in the N₀ and N₁₅₀ treatments. On the other hand, the increased grain N concentration in N₂₀₀ suggest that the additional amount of N added might favoured enhanced N partitioning from source to sink. Our findings are in conformity with Weerakoon *et al.*, (2005) who reported that rice grain N concentration increased at highest dose of nitrogen as a result of improved N partitioning. Overall, the results of OTC studies suggest that excessive N fertilization above the current recommendation is necessary for the future high CO₂ environments.

This thesis has evaluated two systems of rice cultivation for their carbon sequestration potential involving the soil organic carbon fractions. The data from this study provided insights of carbon sequestration in rice ecosystem and brought out salient features associated with SRI system of rice cultivation that can be explored further. The SRI system has an unique advantage for favoring the accumulation of passive pools of soil carbon as measured by humic acid, fulvic acid and glomalin. Despite such improvement, the SOC did not get altered suggesting that carbon dynamics is quite complex and short term manipulation may not yield fruition in enhancing soil carbon

stock. Further, the biochemical and physiological changes in rice plants exposed to elevated $[\text{CO}_2]$ has shown close co-ordination between C and N assimilatory pathways. Though CO_2 enrichment is considered as a source for photosynthesis, this may not enhance the productivity unless the crop is nourished with excess dose of nitrogen.

Overall, the data have shown clear evidence that SRI system of rice cultivation has twin advantages of improving the passive pools of soil carbon besides circumventing methane emission. These processes may be construed as factors responsible for carbon sequestration, thus considering as a potential strategy for mitigating the ill-effects of global warming.

Summary and Conclusions

CHAPTER 6

SUMMARY AND CONCLUSIONS

The carbon sequestration pattern and methane mitigation potentials in two distinct systems of rice cultivation were assessed besides studying the response of rice under elevated levels of CO₂. This study hypothesized that System of Rice Intensification (SRI) which encompasses production of fibrous profusely branched root system, larger biomass productivity and exclusion of submergence that prevents methane emission will result in accumulation of active (biomass carbon) and passive (humic acid, fulvic acid, humin, glomalin) pools of C and facilitates in carbon sequestration. In addition, the OTC study hypothesized that availability and assimilation of nitrogen is a critical factor to rice plants exposed to elevated levels of CO₂ for harnessing the benefits of CO₂ fertilization effect. A brief summary of results obtained and conclusions drawn are presented hereunder.

6.1. Active and passive pools of carbon

The soil carbon sequestration pattern of conventional and SRI systems of rice cultivation with varying levels of nitrogen were assessed based on the active and passive pools of soil carbon.

- The active pool of carbon (microbial biomass carbon) was consistently higher in SRI by 13.3 per cent and 16.3 per cent summer and *kharif*, seasons, respectively, in comparison to conventional system of rice cultivation. This is attributed to the alternate wetting and drying, cono-weeding besides highly branched fibrous root system in rice which facilitates intense microbial activities in rice soils.
- The passive pools of carbon comprising of humic acid, fulvic acid and glomalin were found to be higher in SRI system of rice cultivation suggesting that this system favours enhanced humification and microbial processes that assist in retention of long lived pool which plays an important role in carbon sequestration of any agro-ecosystem.
- Despite the fact that both active and passive pools of carbon were consistently higher in SRI, the SOC has shown lower values throughout the experimental period. This indicates partial aerobic system that may have

facilitated rapid decomposition and retention of relatively lower amounts of SOC in SRI. The data have been further confirmed with laboratory studies where CO₂ emission was higher when the soils were kept under alternate wetting and drying.

- Nitrogen fertilization has linearly and significantly enhanced the active and passive pools of carbon while diminishing the SOC.

6.2. Greenhouse Gases Emission (CH₄ and CO₂)

- The SRI system of rice cultivation had lower methane emissions in both summer (31.8 kg ha⁻¹) and *kharif* (37.7 kg ha⁻¹) seasons, respectively. The data clearly demonstrated that SRI has an unique advantage of lowering methane emissions and could be a potential strategy in minimizing GHG emissions. Such reduction is attributed to the four cycles of cono-weeding, alternate wetting and drying and stimulated methanotrophs population. A strong positive correlation of methanogens, total anaerobes with methane emission was observed. On the other hand, the observations revealed a negative correlation between sulphate reducing bacteria and methane emission.
- In contrast to the methane emission, the SRI had higher CO₂ emission values in both summer (199 kg ha⁻¹) and *kharif* seasons (131 kg ha⁻¹). Such trend is caused by rapid decomposition in SRI which closely coincided with retention of less SOC.
- Incremental levels of N fertilization favoured both CH₄ and CO₂ emissions irrespective of seasons.

6.3. Soil biological changes

- The SRI recorded consistently higher numbers of bacterial, fungal and actinomycetes populations than conventional system. This is evident that SRI system which makes the soil more aerobic besides producing large root system have favoured for enhanced microbial activities. This is further supported by soil enzymatic activities particularly dehydrogenase which is a measure of total biological activity.

- Nitrogen fertilization was found to enhance the soil microbial population and soil enzymatic activities irrespective of the systems of rice cultivation.

6.4. System based carbon sequestration pattern

- The carbon sequestration pattern of conventional and SRI systems was assessed in system perspective considering the carbon flow in above ground (carbon in plant biomass) and below ground (root carbon and soil organic carbon) within the two distinct systems of rice cultivation. Among the systems, the SRI had lower above ground and below ground carbon stock to the level of 9.5 and 7.9 per cent, 10.0 per cent and 7.0 per cent, respectively during summer and *kharif* seasons in comparison to conventional system.
- Despite the fact that the rice plants in SRI were superior in terms of highest number of tillers, productive tillers and root biomass per hill, our observations on these parameters including the above ground and below ground biomasses per unit area basis revealed a different scenario. The conventional system recorded the higher number of productive tillers and root biomass per m² than SRI.
- Our data do not support a hypothesis that SRI systems of rice cultivation produce more biomass both above ground and below ground that accumulate carbon. During the experimentation, it was obvious that the reduction in population as a consequence of wider spacing i.e., 25 cm x 25 cm to the tune of 68 per cent in comparison to conventional system which is hardly compensated by the enhanced production of productive tillers per hill and biomass. This experiment brings out a strategy in which modification of spatial geometry in the existing system of SRI will provide twin advantage of higher productivity in conjunction with greater amounts of carbon sequestration.
- Incremental levels of nitrogen fertilization had increased both the above ground and below ground carbon stock but the increase was linear and conspicuous for above ground carbon stock, while such response was less pronounced in below ground carbon stock.

6.5. Response of rice to elevated CO₂ levels

- The carbon assimilation in rice plant was indirectly measured in terms of soluble protein which constitutes more than 50 per cent of the RUBISCO. The results indicated that elevated CO₂ levels commensurate with photosynthetic activities. Since, rice being a C₃ plant it is quite possible that elevated CO₂ levels favour carbon assimilation under the non-limiting nitrogen fertilization.
- The key enzymes involved in N assimilation pathway namely NRase, GS, GOGAT were shown to be higher at the vegetative phase under elevated [CO₂] where plants carry out active photosynthesis, while such response was declining with the progression of crop growth due to the fact that the metabolites should have been remobilized towards grain filling rather than retained in the leaf itself.
- The elevated [CO₂] viz., 550 and 750 $\mu\text{mol mol}^{-1}$ significantly increased the SMBC and DOC throughout the cropping period. However, the elevated CO₂ levels did not significantly influence the soil organic carbon (SOC) content regardless of stages of observation. Nitrogen fertilization significantly decreased the SOC at all levels of CO₂ concentrations.
- Elevated CO₂ levels favour methane emission as a result of enhanced carbon assimilation and production of energy rich root exudates that stimulate microbial activities in the rhizosphere. Consistent with the observed increases in methane flux, the enumeration of methanogens showed a significant increase under elevated [CO₂]. In addition, the methanotrophs population was also significantly highest under elevated CO₂ levels regardless of stages of observation. Incremental levels of nitrogen addition significantly increased the methanogens but decreased the methanotrophs population regardless of CO₂ concentrations or stages of observation.
- Rice plants exposed to elevated [CO₂] recorded higher above ground and below ground biomasses as a result of 'CO₂ fertilization effect'. Such response is obvious only when N fertilization is done excessively by 25 per cent of the recommended dose of nitrogen.

- The rice grain quality in terms of nitrogen concentration was significantly decreased under elevated $[\text{CO}_2]$. Without adequate N, the rice plants grown at high CO_2 became inferior to plants grown at ambient CO_2 with respect to grain quality.

The field experiments and laboratory studies conducted to assess the carbon sequestration potentials of SRI and conventional systems of rice cultivation has clearly demonstrated that SRI has a unique advantage in improving the passive pools of soil carbon besides minimizing methane emission. Despite the fact that SRI could be one of the potential methane mitigation strategies, the productivity per unit area was observed to be lower than conventional system of rice cultivation. This necessitates modifying the spatial geometry of SRI and manuring practices to gain the twin advantage of enhanced productivity while circumventing methane emission from rice fields. The OTC experiments on elevated $[\text{CO}_2]$ have unequivocally demonstrated that CO_2 enrichment will favourably improve the productivity and nutritional quality only when the rice plants are nourished with excessive N fertilization above the current recommendation.

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Appendices

APPENDIX - 1

Weather conditions prevailed during the experimental period

Standard weeks	Temperature (°C)		Relative humidity (%)		Wind speed (km/hr)	Sunshine (Hrs)	Rainfall (mm)	Evaporation (mm)
	Max	Min	07.22 hrs	14.22 hrs				
Field experiment during summer season (2011)								
Jan 15 - 21	30.2	18	86.9	40.3	4.7	8.3	0	9.9
Jan 22 - 28	29.5	19.6	86.3	40.7	7.6	9.2	0	4.3
Jan 29 - Feb 04	30.9	17.4	86.4	32.4	5.9	10	0	4.7
Feb 05 - 11	31.9	16.5	85.4	31.7	4.1	10	0	4.5
Feb 12 - 18	31.7	17	87.6	35.6	4.5	9.7	0	5.3
Feb 19 - 25	32.4	21.5	87.9	51.4	4.6	7.1	46.4	4.5
Feb 26 - Mar 04	30.9	19.7	89.6	41	5.1	8.5	79.2	4.8
Mar 05 - 11	33.7	19.3	88.1	29	3.7	10.1	0	5.2
Mar 12 - 18	33.7	21	86.4	29	5.1	9.6	0	5.8
Mar 19 - 25	34.1	21.2	87.6	39.7	3.7	8.6	0	5.4
Mar 26 - Apr 04	33.8	22.5	91.1	44.3	3.5	8	23.6	4.6
Apr 02 - 08	33.9	22.8	85	43.9	3.9	7.7	2.8	4.9
Apr 09 - 15	34.4	23.7	84.9	44.3	4.4	8.2	0.2	5.1
Apr 16 - 22	34	22.4	90.4	47.9	4.3	7.3	132.6	4.9
Apr 23 - 29	31.4	22.7	94	59.9	3.7	6.9	33.2	3.8
Apr 30 - May 06	33.3	23.2	92.4	59.9	4.3	8.2	23.2	4.7
May 07 - 13	33.6	21.2	88.1	50.7	4.2	10.5	0	5.2
May 14 - 20	34.6	23.7	89.6	54.3	3.6	8	0	5

Standard weeks	Temperature (°C)		Relative humidity (%)		Wind speed (km/hr)	Sunshine (Hrs)	Rainfall (mm)	Evaporation (mm)
	Max	Min	07.22 hrs	14.22 hrs				
Field experiment during <i>kharif</i> season (2011)								
June 18 - 24	30.6	21.6	83.6	52.3	8.5	7.7	20.5	4.9
June 25 - July 01	31.4	22.9	86	50.7	7.8	5.1	4.1	4.8
July 02 - 08	31.7	23.1	86	49.1	8.7	4.8	0.4	5.3
July 09 - 15	31.6	23.4	84	61.7	9	9.1	17.6	5.5
July 16 - 22	30.2	23.3	75.3	60.1	14.1	2.6	11.6	5.7
July 23 - 29	30.8	22.8	85.7	53.3	9.4	4.4	1.5	5.0
July 30 - Aug 05	30.5	23.2	76.9	58.1	10.8	2.9	0.8	4.6
Aug 06 - 12	32.1	22.7	91.3	50.9	8.0	4.8	0.0	5.6
Aug 13 - 19	31.5	22.6	92.6	57.7	5.3	3.5	4.0	4.2
Aug 20 - 26	30.8	22.5	91.6	60.4	5.9	5.9	1.5	3.8
Aug 27 - 02	30.3	23.1	80.6	64.9	13.4	5.8	12.1	6.1
Sep 03 - Sep 09	26.6	22.8	85.7	59.3	12.0	7.2	3.8	5.6
Sep 10 - 16	30.9	22.3	91.4	62.0	6.6	5.2	33.5	4.8
Sep 17 - 23	31.7	21.9	88.6	50.7	7.8	7.0	0.0	5.5
Sep 24 - 30	32.5	21.7	88.0	54.4	5.0	9.2	0.0	5.6
Oct 01 - Oct 07	33.4	22.5	88.0	48.6	4.6	9.2	0.0	5.5
Oct 08 - 14	33.0	22.9	87.4	54.1	4.6	7.2	71.5	5.0
Oct 15 - 21	31.6	22.6	94.7	60.6	3.6	6.7	111.4	3.8
Oct 22 - 28	29.6	22.6	90.7	66.7	4.6	4.5	39.0	3.7

APPENDIX – 2

Media composition for enumeration of aerobic microorganisms

1. Composition for Nutrient agar medium

Glucose	:	5g
Peptone	:	5g
Beef extract powder	:	3g
NaCl	:	5g
Agar	:	20g
pH	:	7 – 7.2
Distilled water	:	1 lt

2. Composition for Rose bengal agar medium

Glucose	:	5g
Peptone	:	5g
KH ₂ PO ₄	:	1.5g
MgSO ₄	:	0.5g
Rose Bengal	:	33 mg
Agar	:	20g
Streptomycin sulphate	:	3ml
pH	:	6 – 6.3
Distilled water	:	1 lt

3. Composition for Kenknight's agar medium

Glucose	:	5g
K ₂ HPO ₄	:	1g
NaNO ₃	:	1g
KCl	:	0.10g
MgSO ₄	:	0.10g
Agar	:	20g
pH	:	7 – 7.5
Distilled water	:	1 lt

APPENDIX – 3

Media composition for enumeration of methanotrophs

1. Noble Agar Medium (NSM) (per litre)

Sodium nitrate	:	1 mM
Potassium sulphate	:	1 mM
Magnesium sulphate	:	0.15 mM
Calcium chloride	:	47.6 μ M
Potassium di hydrogen phosphate	:	3.9 mM
Di sodium hydrogen phosphate	:	6 mM
Zinc sulphate	:	2 μ M
Manganous sulphate	:	1.6 μ M
Boric acid	:	6 μ M
Sodium molybdate	:	0.4 μ M
Cobaltous chloride	:	0.4 μ M
Potassium Iodide	:	1 μ M
Ferrous sulphate	:	40 μ M
Agar	:	20 g
pH	:	7.0

2. Phosphate Buffer (for serial dilution)

A. Sodium hydrogen phosphate	:	10mM
B. Sodium di hydrogen phosphate	:	10 mM

Mix 61 ml of solution A with 39 ml of solution B to make 100 ml of the phosphate buffer

APPENDIX – 4

Media composition for enumeration of anaerobes in soil

1. Dilution of the soil sample:

Dilution medium

Sodium bicarbonate	:	0.5%
Sodium carbonate	:	1.0%
Resazurin	:	0.0001%

2. Dilution buffer:

Glass distilled water	:	500 ml
General salt solution*	:	500 ml
Sodium chloride solution	:	75 ml (293 g/ l)
Resazurin	:	1 ml
Cysteine hydrochloride	:	0.5 g

***Composition of general salt solution (g/l):**

Potassium chloride	:	0.67
Magnesium chloride	:	11.20
Ammonium chloride	:	1.00
Calcium chloride	:	0.28

3. Media for total anaerobic bacteria:

Modified Hungate's medium (g/l):

Potassium dihydrogen phosphate	:	0.02
Dipotassium hydrogen phosphate	:	0.03
Magnesium sulphate	:	0.01
Calcium chloride	:	0.01
Ammonium sulphate	:	0.10
Sodium chloride	:	0.02
Cysteine HCl	:	0.02
Sodium bicarbonate	:	0.50

Resazurin	:	0.0001
Glucose	:	0.50
Maltose	:	0.25
Cellobiose	:	0.25
Agar	:	0.20
Vitamin solution*	:	1.0 ml
Trace element solution**	:	1.0 ml
pH	:	7.0

***Vitamin solution**

Biotin	:	10 mg
Nicotinic acid	:	100 mg
p-amino benzoic acid	:	50 mg
Thiamine	:	100 mg
Pantothenic acid	:	50 mg
Pyridoxamine	:	250 mg
Cobalamin	:	50 mg
Distilled water	:	1000 ml

****Trace element solution**

12.5 N HCl	:	4.0 ml
FeCl ₃ . 4 H ₂ O	:	2000 mg
ZnCl ₂	:	70 mg
MnCl ₂ . 4 H ₂ O	:	100 mg
CoCl ₂ . 6 H ₂ O	:	190 mg
CuCl ₂ . 2 H ₂ O	:	17 mg
NiCl ₂ . 6 H ₂ O	:	24 mg
Na ₂ MoO ₄ . 2 H ₂ O	:	36 mg

$\text{Na}_2\text{SeO}_4 \cdot 5 \text{H}_2\text{O}$	:	39 mg
$\text{Na}_2\text{WO}_4 \cdot 2 \text{H}_2\text{O}$	:	49 mg
Distilled water	:	1000 ml

Vitamin and trace element solution was prepared, filter sterilized and stored in sealed sterile vials at 4°C and added to the medium just before plating.

4. Medium for sulphate reducing bacteria

Solution 1:

Distilled water	:	960.0 ml
KH_2PO_4	:	0.5 g
NH_4Cl	:	0.5 g
Na_2SO_4	:	3.0 g
$\text{MgSO}_4 \cdot 6 \text{H}_2\text{O}$	:	0.5 g
$\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$	:	0.1 g
Sodium lactate (50% solution)	:	4.0 ml
Yeast extract	:	1.0 g
Agar	:	12.0 g

Adjust the pH to 7.4

For MPN technique the same media was used without agar

Solution 2:

Distilled water	:	19.0 ml
Ethanol	:	1.0 ml

Autoclaved in sealed bottles with nitrogen gas phase

Solution 3:

Distilled water : 10.0 ml

FeSO₄.7 H₂O : 0.5 g

Acidified this solution with diluted H₂SO₄

Solution 4:

Distilled water : 10 ml

Ascorbic acid : 0.1 g

Sodium thioglycollate : 0.1 g

Adjusted pH to 7.4

Autoclaved in sealed bottles or tubes under nitrogen gas atmosphere

5. Medium for methanogenic bacterial (g/l)

NH₄Cl : 1.0

K₂HPO₄. 3 H₂O : 0.4

MgCl₂. 6 H₂O : 0.1

Trypticase/ tryptone : 2.0

Yeast extract : 2.0

Calcium acetate : 10.0

Resazurin : 0.001

Agar : 20 g

H₂O : 1000ml

APPENDIX – 5

Maximum and minimum air temperature variations in
OTC's and ambient conditions

