CHARACTERISATION OF BIOLOGICAL POOLS OF CARBON, NITROGEN AND PHOSPHOROUS IN SOILS OF RICE ECOSYSTEMS IN MEGHALAYA

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in
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Dedication

This thesis is dedicated to
my
beloved parents
and
family members

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Christy Berylnight K. Sangma

ABSTRACT

Soil ecosystems are complex and biogeochemistry of soils is governed largely by the functioning of soil biota community through their control over the biological pool of every elemental cycle. Biological pools of carbon (C), nitrogen (N) and phosphorus (P) in soils of hill rice ecosystems in North East India are not yet characterized. This study characterized an array of biological parameters of C, N and P in soils of three common rice ecosystems viz. slope land, upland terrace and lowland. Soil samples (0-15 cm depth) were collected from six different sites located in two villages (Saiden and Kyrdemkulai) in post-summer and in post-winter seasons. The soil of each site differed significantly between seasons in terms of soil microbial biomass C, N and P, total and dissolved organic carbon, extractable organic nitrogen, potentially mineralizable nitrogen, basal and substrate induced respiration, total N and P, available N and P, soil dehydrogenase and phosphatase activities ($P < 0.01$, n=20 as determined by paired t-test within a site) and these parameters were strongly influenced by soil moisture content. Pair-wise correlation matrix analysis revealed that biological parameters of C, N and P were strongly influenced by each other (correlation coefficient $r \geq 0.36$ at $P<0.05$, or ≥ 0.46 at $P<0.01$, n=30). Principal component analysis (PCA) performed season-wise considering the biological parameters as defined variables indicated that rice fields were grouped according to ecosystem type and soil moisture status, and such effects overrided the impacts of site differences in biological pools of C, N and P. Moisture content in soils observed to be a critical variable in hill rice ecosystems that control the size and dynamics of biological pools of C, N and P and the interrelationships among these parameters. Overall, it can be concluded that C and N components of soils in lowland and stabilized upland terrace rice ecosystems seem to be self-sustained, but the major limiting factor was availability of P.

CONTENTS

Chapter No.	Title		Page No.
	Certificate I		
	Certificate II		
	Dedication		
	Acknowledgement		
	Abstract		
	Contents		i-ii
	List of Tables		iii
	List of Figures		iv
	Abbreviations		v-vi
1.0	**Introduction**		1-3
2.0	**Review of Literature**		4-25
	2.1	Rice Ecosystems	4-6
	2.2	Nutrient management in rice soils of hill agriculture	6-8
	2.3.1	Biological pools of carbon in agricultural soils	8-10
	2.3.2	Biological pools of carbon in rice soils	10-11
	2.4.1	Biological pools of nitrogen in agricultural soils	12-13
	2.4.2	Biological pools of nitrogen in rice soils	13-14
	2.5.1	Biological pools of phosphorus in agricultural soils	14-16
	2.5.2	Biological pools of phosphorus in rice soils	16-17
	2.6	Seasonal dynamics of pools of carbon, nitrogen and phosphorus in soil	17-20
	2.7	Interactions among carbon, nitrogen and phosphorus pools	20-24
	2.8	Enzyme activity	24-25
3.0	**Method and Materials**		26-37
	3.1	Description of the sampling site	26
	3.2	Climate and soil	26
	3.3	Soil sampling and processing	27
	3.4	Soil analysis	29
	3.4.1	Soil moisture content	29
	3.4.2	pH	29
	3.4.3	Total Organic Carbon (TOC)	29
	3.4.4	Dissolved Organic Carbon (DOC)	29-30
	3.4.5	Total Nitrogen (TN)	30
	3.4.6	Extractable Organic Nitrogen (EON)	30-31
	3.4.7	Soil Available Nitrogen (Avl-N)	31
	3.4.8	Total Phosphorus (TP)	31-32
	3.4.9	Soil Available Phosphorus (Avl-P)	32
	3.5	Soil Biochemical Analysis	32
	3.5.1	Microbial Biomass Carbon (MBC)	32-33

Chapter No.	Title		Page No.
	3.5.2	Microbial Biomass Nitrogen (MBN)	33
	3.5.3	Microbial Biomass Phosphorus (MBP)	33-34
	3.5.4	Potentially Mineralizable nitrogen (pMN)	34
	3.5.5	Basal Respiration (BAS)	34-35
	3.5.6	Substrate Induced Respiration (SIR)	35
	3.5.7	Dehydrogenase Activity (DHA)	35
	3.5.8	Phosphatase Activity (PHA)	36
	3.6	Statistical Analysis	36
	3.6.1	Univariate statistics	36
	3.6.2	Multivariate statistics	36-37
4.0	**Experimental Findings**		38-52
	4.1	Soil particle distribution, moisture content, pH and C:N ratio	38-39
	4.2	Soil carbon fractions	39-41
	4.3	Soil nitrogen fractions	41-44
	4.4	Soil phosphorous fractions	44
	4.5	Microbial biomass C, N and P to total C, N and P in soils	44-47
	4.6	Soil enzyme activities	48
	4.7	Comparison of rice ecosystems in terms of soil C, N and P fractions	48-51
	4.8	Relationship among various fractions of soil C, N and P	51 - 52
5.0	**Discussion**		57-65
6.0	**Summary**		66-69
7.0	**Conclusion**		70
	Bibliography		71-98
	Appendix		i-iv
	Appendix 1.1	Principal component analysis performed using various fractions of C, N and P as defined variables in post-summer	i-ii
	Appendix 1.2	Principal component analysis performed using various fractions of C, N and P as defined variables in post-summer	iii-iv

LIST OF TABLES

Table No.	Titles	Page No.
Table 3.1	Agricultural management practices at soil sampling sites	28
Table 4.1	Some soil properties of sampling sites under different rice ecosystems	40
Table 4.2	Organic carbon fractions and basal respiration in soils of rice ecosystems as influenced by seasons	42
Table 4.3	Nitrogen fractions in soils of rice ecosystems as influenced by seasons	45
Table 4.4	Phosphorus fractions in soils of different rice ecosystems as influenced by seasons	46
Table 4.5	Contribution of microbial biomass carbon, nitrogen and phosphorus to total soil carbon, nitrogen and phosphorus in soils of different rice ecosystems	48
Table 4.6	Pair-wise relationship between parameters of carbon, nitrogen and phosphorus in rice ecosystems during post summer season	55
Table 4.7	Pair-wise relationship between parameters of carbon, nitrogen and phosphorus in rice ecosystems during post winter season	56

LIST OF FIGURES

Figure No.	Title	Page No.
Fig. 4.1	Induced soil respiration due to addition of glucose in soils of different rice ecosystems as influenced by seasons. Within a site, values that differ significantly are followed by different letters at $P \leq 0.01$. Amount of CO_2-C produced inside incubation vessels during 7 days of incubation was trapped in NaOH solution and data used for calculation of glucose induced soil respiration.	43
Fig.4.2	Dehydrogenase activity in soils of different rice ecosystems as influenced by seasons. Within a site, mean values (columns) with different letters were significantly different ($P<0.05$).	49
Fig. 4.3	Phosphatase activity in soils of different rice ecosystems as influenced by seasons. Within a site, mean values (columns) with different letters were significantly different ($P<0.05$).	50
Fig. 4.4	Comparison of variability (in terms of pH and various fractions of C, N and P, and soil enzyme activities) among different rice ecosystems in post-summer performed by principal component analysis. Data were square root transformed and normalized to eliminate the effects of units. Principal axes 1 and 2 expressed variability of 48.7% and 16.2%, respectively. Ellipses represent superimposed hierarchical clusters (Euclidean distance 5.7) based on Bray-Curtis similarity matrix, deduced using group-average linking incorporating similarity of profile (SIMPROF) at 95% confidence limit.	53
Fig.4.5	Comparison of variability (in terms of pH and various fractions of C, N and P, and soil enzyme activities) among different rice ecosystems in post-winter performed by principal component analysis. Data were square root transformed and normalized to eliminate the effects of units. Principal axes 1 and 2 expressed variability of 49.5% and 20.6%, respectively. Ellipses represent superimposed hierarchical clusters (euclidean distance 5.7) based on Bray-Curtis similarity matrix, deduced using group-average linking incorporating similarity of profile (SIMPROF) at 95% confidence limit.	54

ABBREVIATIONS

%	Per cent
µ	Micron
µg	Microgram
µg g^{-1}	Microgram per gram
µM	Micro-molar
ºC	Degree celsius
Avl-N	Available nitrogen
Avl-P	Available phosphorous
BAS	Basal respiration
C	Carbon
cm	Centimetre
CO_2	Carbon dioxide
DHA	Dehydrogenase activity
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
D/W	Drying and wetting
EON	Extractable organic nitrogen
g	Gram
h	Hour
ha	Hectare
kg	Kilo-gram
l	Litre
LFOM	Light fraction organic matter
m	Metre
M	Molar
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MBP	Microbial biomass phosphorous
mg	Milligram
Min	Minute
mm	Millimetre
msl	Mean sea level
N	Normality
nm	Nanometer
PCA	Principal component analysis
Pg	Pico-gram
PHA	Phosphatase activity
pMN	Potentially mineralizable nitrogen
P_i	Inorganic phosphorus
P_o	Organic phosphorus
POM	Particulate organic matter
POMC	Particulate organic matter carbon
r	Correlation coefficient
rpm	Rotation per minute

SIR	Substrate induced respiration
SMB	Soil microbial biomass
SOM	Soil organic matter
SPSS	Statistical package for Social Science
sq km	Square Kilo-meter
SR	Soil respiration
SRR	Soil respiration rate
SYI	Sustainable yield index
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
$w\ v^{-1}$	volume per weight
wt	Weight
WSA	Water stable aggregation

CHAPTER 1

Introduction

Almost half of the world population is dependent on rice (*Oryza sativa* L.) and this population entirely depends on rice systems as their main source of employment and income (Nguyen and Ferrero, 2006). The three rice production systems viz. irrigated rice; rainfed lowland rice and rainfed upland rice occupy 93% of the world's rice area and produce 96% of the total rice (IRRI, 1993). Each year, 50 million new people mostly rice eaters are added to Asia. To feed them, the world must increase its rice output between now and 2020 by one-third more than what is grown and eaten today (Rice and Hope, IRRI, 2010). This must be achieved without the mass use of chemical fertilizers and pesticides, which may cause environmental pollution and negatively influence human health. And to do this, we must understand the biogeochemistry of rice ecosystems so that nutrient supply to the crops can be managed efficiently under rice based cropping system.

Soil ecosystems are complex and this ecosystem functioning is governed largely by soil microbial dynamics (Schimel, 2007). Soil functions such as nutrient cycling, residue decomposition, and soil structure formation are regulated by soil fauna and microbiota and thereby, they regulate the productivity and health of agricultural systems (Kennedy and Papendick, 1995; Pankhurst *et al*., 1996). Carbon (C) is the key attribute of soil quality (Christensen and Johnston, 1997; Carter, 2002) because it influences nutrient cycling, soil structure, water availability, and other important soil properties (Arshad and Cohen, 1992; Doran *et al*., 1998; Yakovchenko *et al*., 1998). Carbon and Nitrogen (N) are known to interact closely during residue decomposition by heterotrophic soil microbiota (Sall *et al.,* 2007) and hence, assessment of biological pools of C and N are important in understanding the functioning of an ecosystem.

Soil microbiota governs the available pools of nutrients in rice ecosystems. Microbial biomass nitrogen (MBN) contributes the largest fraction of the

biologically active N pool in soil (Jenkinson and Parry, 1989) and the content is depended on quantity, quality, and distribution of carbon inputs in soils that may vary with times and depth (Kaiser and Heinemeyer, 1993). Microbial biomass carbon (MBC) as a relatively labile fraction of organic matter is a key site for mineralization of organic P in soils and thus, is a central feature in the P cycle (Brookes *et al*., 1984). Phosphorus dynamics in soil and maintenance of its adequate supply are important for sustainability of native and agricultural ecosystems. Soil P transformations are primarily mediated by microbial activity, which in turn is influenced by a combination of factors including plant species, soil type and environmental conditions. Microbial biomass phosphorus (MBP) is considered an important source of P for plant uptake (Macklon *et al.*, 1997) in P-fixing soils (Ayaga *et al*., 2006). The quantity and quality of soil organic matter provides an important diagnostic link between management and sustainability of soil function. MBC is a sensitive indicator of changes in the quality and quantity of soil organic matter (SOM). It responds more rapidly than total SOM to changes in organic inputs to the soil or in soil management, and differences in MBC can be detected before differences can be measured in total SOM (Carter, 1986; Powlson *et al*., 1987). Overall, microorganisms have a fundamental role in the biogeochemical cycles of the elements and in the formation of soil structure (Roldán *et al*., 1994), such that it is widely accepted that a high level of microbial activity is necessary for the maintenance of an adequate quality of soil.

Nutrient use efficiency in acidic rice soils of North East India is very low and the depletion of organic matter in top soils under hill slopes is at a very high rate due to excess runoff. Meghalaya, one of the states of North East India, consisted of hilly terrain and acidic soils and receives high annual rainfall. Rice is the main crop for the people of Meghalaya and they cultivate it under three distinct ecosystems viz. upland terrace, lowland and slope land (Tripathy *et al*., 2007). These rice ecosystems are likely to be different from each other in terms of ecosystem functioning because of differences in soil type, water content, slopes, aboveground and belowground biomass and anthropogenic activities. Understanding the C, N and P processes operating under each rice ecosystem, it is

important to study the biogeochemistry of C, N and P. The biological pools of C, N, and P in soils of rice ecosystems under hill agriculture in the NE region of India are not known. Though, in past, several studies determined the C and N dynamics in rice soils under different nutrient management regimes; but remained confined to the inorganic pools of C and N in rice soils. Currently, the efficacy of various nutrient management approaches in soils of NE hill states of India are compared and evaluated on the basis of inorganic pools of C, N and P; but not on the basis of comparative evaluation of biological pools of C, N, and P. Understanding the biological pools of C, N, and P in soils of rice ecosystems are necessary for effective management of plant essential nutrients through better management of biological components. Knowledge on these aspects will certainly help in managing or altering nutrient availability and supplying capacity of soils through effective management of available natural resources.

Keeping the above points in mind, this research is proposed based on the null hypothesis is that the three common rice ecosystems in the hill states of NE India are similar in terms of biological pools of C, N and P and hence, biogeochemistry of these ecosystems are not different. To prove this null hypothesis the following objectives are formulated:

1. **To assess biological pools of carbon, nitrogen, and phosphorus in soils of direct-seeded hill slopes, transplanted upland terrace, and lowland rice fields.**

2. **To determine relationship among various parameters of biological pools of C, N, and P.**

CHAPTER 2

Review of Literature

"***In order to effectively feed the fertile population from the infertile soil in a fragile world***" (Borlaug and Dowswell, 1993), it is essential to develop improved technologies that enhance crop productivity through improved resource use efficiency and reduction in losses. To achieve this goal, we must develop thorough understanding of soil biogeochemistry in agro-ecosystems. Biological component of every elemental cycle is crucial as it acts as a driving force in controlling the mineralization and immobilization dynamics. Rice ecosystems are the major concern because of low nutrient use efficiency and high nutrient losses especially under rainfed conditions. Hence, existing knowledge on biological pools of C, N and P in rice ecosystems will be reviewed below.

2.1 Rice ecosystems

Rice is the staple food for the half of world's population. It is grown in agro-ecosystems that range from uplands to the flood prone and coastal wetlands. Rice is the major cereal crop for the people of the N E India and rice cultivation is practiced in three distinct ecosystems. They are upland terrace, lowland and slope lands (Tripathi *et al.,* 2007). These rice based ecosystems are likely to be different from each other in terms of ecosystem functioning.

Waterlogged (lowland) rice ecosystems harbour a unique microbial ecology. They remain flooded during a major part of the crop growing period and are predominantly anaerobic. These ecosystems are different from upland soils in several physicochemical and biological properties (Leisack *et al*., 2001; Adhya and Rao, 2005). Considerable amounts of aquatic biomass are likely to be produced phototrophically in the floodwater (Roger, 1996) and even chemo-

heterotrophically in the presence of inorganic electron donors and CO_2 along the redox gradients in waterlogged rice soils (Revsbech *et al*., 1999). Thus, flooded soils represent a changed dynamics of microbial biomass and activity as compared to upland soils. About 75% of the global rice volume is produced in the irrigated lowlands (Maclean *et al*., 2002). In eastern India, where approximately 80% of the 20 million hectares of rice is grown in rainfed lowlands (CRRI, 1990; Widawsky and O'Toole, 1990), there have been low but continuous increases in rice yields (David and Otsuka, 1994). The factors known to affect the indigenous soil nutrient supply in irrigated rice systems are soil drying, length of fallow, crop rotation, and residue management (Witt *et al.*, 1998). In the principal rainfed lowland rice regions, farmers are very poor, tend to be risk-averse and grow traditional varieties using traditional methods. Many of these cultivars are sensitive to submergence and drought, and are susceptible to diseases (Mackill, 1986; Mackill *et al*., 1996). Rainfed lowland rice farmers apply very low rates of fertilizer, probably because the potential yield is at great risk in the event of a flood or drought (CRRI, 1990). In general, moisture stress is most damaging near the reproductive stages of the rice crop (Widawsky and O'Toole, 1990). Weeds are increasingly among the most severe biotic constraints, while losses to pests and diseases are seen to be less severe in rainfed systems than in irrigated systems (David and Otsuka, 1994; Widawsky and O'Toole, 1990).

Upland rice is grown as a subsistence crop under rainfed conditions with no fertilizer inputs. It has traditionally been grown under slash-and-burn systems with long fallows, which restore soil fertility and reduce insect and weed pressure. However, increasing population density and government policies aimed at reducing the area under slash-and-burn have reduced fallow cycles to as little as two or three years between rice crops. Sloping fields, combined with heavy tillage, strong winds, and lack of ground cover, lead to extensive erosion and rain-runoff events. Changes in tillage, residue, and rotation practices induce major shifts in the number and composition of soil fauna and flora, including both pests and beneficial organisms (Bockus and Shroyer, 1998; Andersen, 1999). Productivity and qualities of the agricultural soils depend on the microbial

functionality which generally decreases with environmental stress (Atlas *et al.*, 1991, Pankhurst *et al*., 1996).

In hill agriculture where the places are sloppy, flat terraces are essential for cultivation of rice. But this mechanical terracing is an aggressive strategy that results physical alterations in the soil, such as compaction. The surface horizons can disappear or suffer serious disruption, resulting in the loss of a large part of the microbial population and thus, its activity. Soil management is sustainable only when the quality is maintained or improved (Garcia *et al*., 1997). Bastida *et al.* (2007) studied the influence in the long-term (13 years) of two types of physical management (terracing and strip planting) in the reforestation of a mountainside, together with the addition of an organic amendment. The results indicate that terracing in semi-arid, if not accompanied by the addition of organic matter, can have a negative effect on soil microbiological and biochemical properties.

Agricultural use of soil can affect its chemical, physical and biological properties. Comparison of soils between the natural vegetation and the cultivated top soils has revealed that prolonged agricultural land use can alter the magnitude, diversity, and spatial variability of some soil properties, mostly those related to fertility (Paz-Gonzalez *et al*., 2000). Continuous cultivation with frequent tillage results in a rapid loss of organic matter (Balesdent *et al*., 1999), and reduces microbial populations, activities and different enzymatic activities (Shepherd *et al.*, 2001; Ibekwe *et al.*, 2002; Carpenter-Boggs *et al*., 2003).

2.2 Nutrient management in rice soils of hill agriculture

Over the coming decades the challenge for agriculture will be to meet the world's increasing demand for food in a sustainable way. Declining soil fertility and mismanagement of plant nutrients have made this task more difficult. The variable moisture regime of rainfed lowlands contributes to their uncertain fertility (De Datta, 1986). Soil-nutrient management is also an important aspect of improving crop management techniques for enhanced productivity through the

adoption of nutrient-efficient rice varieties, improved nitrogen placement methods and the use of appropriate diagnostic tools. In developing countries, harsh climatic conditions, population pressure, land constraints, and the decline of traditional soil management practices have often reduced soil fertility (Stoorvogel and Smaling, 1990; Tandon, 1998; Henao and Baanante, 1999; Bumb and Baanante, 1996). In spite of the rapid growth in the use of chemical fertilizers during the Green Revolution, Asian farmers of the rainfed region still apply low doses of the chemical fertilizers. In hill agriculture for sustainable rice production nutrients are used mostly through organic manures. Average chemical fertilizer usage in India is 170 kg/ha compared to 32 kg/ha in the rainfed region (Hossain and Singh, 1995). The adoption of nutrient responsive high yielding cultivars in rainfed environment is also limited (Kshirsagar and Pandey, 1996). Agriculture production in rainfed ecosystem is risky and climatic variability is one of the major risks in rainfed rice production. Cassman and Pingali (1995) reported that the intensified rice mono-cropping for several years has begun to show a declining trend in rice yields. Imbalanced nutrient management and decreased SOM is the key responsible for the observed declining trend in rice-based cropping systems (Nambiar, 1995; Reddy and Krishnaiah, 1999). Garcia *et al*. (1997a) considered that the labile fraction of SOM, mainly water-soluble C and carbohydrates, could indicate the soil's potential microbial activity, which is sensitive to land use and management practices, and these fractions have been closely linked to soil productivity because of their capacity to supply nutrients for both plants and microorganisms.

The long-term productivity of upland rice can not be sustained with increased cropping intensity using the current management practices as it is totally depend on total rainfall distribution. Avasthe *et al*. (1995) analysed the acidic rice soils of Sikkim for their fertility status and found that the organic matter varies both in the tropical and sub-tropical soils. Most of the soils showed medium to high available N, high available P and medium to high available K status and some soils are well supplied with micro-nutrients. Takahashi *et al*. (2003) investigated short- and long-term effects of rice straw application to paddy

soils on crop growth, mineralization of straw N, and soil N supply under flooded and upland conditions and found that continuous application of rice straw contributed to the improvement of soil fertility and the promotion of growth and N uptake by paddy rice and upland crops, while short-term effects on dry matter production and N uptake by crops were little. Thakuria *et al*. (2009) demonstrated the benefit of the application of bacterial bioinoculants included integrated nutrient management against the use of only inorganic sources of nutrients in acidic soils under rice based cropping system in alluvial soils of Assam; benefits are in terms of higher grain yield, and higher uptake of N, P and Zn, improved physico-chemical and biological properties. Bioinoculants included integrated nutrient management maintains and improved the biological pools of C, N and P by enhancing the soil microbial activity and improved decomposition dynamics and thereby enhance the nutrient supplying capacity of soil for plant growth and development.

2.3.1 Biological pools of carbon in agricultural soils

Soil organic matter (SOM), one of the most important components of an agro-ecosystem can serve as a soil conditioner, nutrient source, substrate of microbial activity, preserver of the environment and major determinant for sustaining agricultural productivity (Schnitzer, 1991). Labile-SOM pools are the fine indicators of soil quality which are influenced by changes in management practices. There is increased recognition that inputs of labile-C substrates to the soil can significantly stimulate SOM decomposition (Dijkstra *et al.*, 2009). In particular, soil is regarded as the most active and dynamic pool of SOM and plays important role in driving soil mineralization processes. This pool is responsible for the decomposition of plant and animal residues and for the immobilization and mineralization of plant nutrients; in short it is responsible for the maintenance of soil fertility (Brookes, 2000).

Maintenance of soil organic C (SOC) is considered essential for long-term sustainable agriculture because declining SOC levels in soil generally leads to

decrease in productivity. Plant roots are an important source of labile C (root exudates and other rhizodeposits (Hutsch *et al*., 2002), and several studies have shown that when plants are present, SOM decomposition can increase up to 380% compared to soil incubations lacking plants (Cheng *et al*., 2009).

Dissolved organic carbon (DOC) represents a small but functionally very important fraction of the SOM (van Hees *et al*., 2005). It is the mobile fraction of SOM (Kalbitz *et al.*, 2000). The DOC pool is extremely dynamic. Majumder *et al.* (2007) reported that some sub-pools of DOC namely very labile-C, mineralizable–C, and POMC were significantly correlated with each other in terms of their contents in soils and with yields of crops and sustainable yield index (SYI) of the 34 years old rice-wheat-jute cropping system. The authors suggested that labile-C fractions can reasonably be used as good indicator for assessing soil in terms of crop productivity. Muller *et al*. (2009) estimated the contribution of ^{13}C-depleted recent plant C to DOC of mor-type organic layers. Results of the field study showed that DOC in the O_a horizon at 5 cm contained only 20±3% of less than six year old C, indicating minor contributions of throughfall, root exudates, and fresh litter to leached DOC. The result give evidence on an apparent exchange of DOC in thick organic layers with litter-DOC being retained and replace by older DOC leached from the large pool of indigenous SOM.

Soil respiration (SR) is a major process controlling carbon loss from terrestrial ecosystems. On a global scale, SR was estimated to produce 80.4 Pg C y^{-1} (pentagram carbon per year) with a range of 79.3–81.8 Pg C y^{-1} (Raich *et al*., 2002), accounting for 60– 90 percent of total respiration of global terrestrial ecosystems (Schimel *et al*., 2001), which is more than 11 times the current rate of fossil fuel combustion (Marland *et al*., 2000). SR is usually understood as evolution of biologically generated CO_2 from the soil surface. The decay of SOM provides the largest CO_2 input into the atmosphere (Schnitzer, 1978). One of the most widespread methods to determine microbial activity is quantifying soil basal respiration. Basal respiration (BAS) is the steady rate of respiration in soil which originates from the turnover of organic matter. The rate of BAS reflects both the

amount and quality of carbon source (Pell *et al*., 2006). Duong *et al*. (2009) investigated the effect of frequent residue addition on C mineralization and N dynamics. The result showed that frequent residue additions increase C mineralization but did not affect N dynamics or the size of the microbial biomass and had little effect on microbial community composition. This suggests that microbes respire more of the added C (and possibly native soil C) per unit biomass but this does not change their N requirement or the microbial community composition. The substrate-induced respiration (SIR) method is based on the detection of the respiratory response of soil microorganisms on supply of glucose. Addition of soluble organic substrates to soil has been shown to either increase or restrict the rate of microbial CO_2–C evolution. Hoyle *et al.* (2008) investigated microbial responses to small glucose-C additions (10–50 μg C g^1 soil) in arable soils either amended or not with cellulose. Findings indicated that the response of the microbial community to small additions of soluble organic C substrate is not consistent and support the premise that microbial response varies between soil type and ecosystems. Besides, root respiration also contributes to soil respiration. The lability is defined with the ease and speed with which it is decomposed by microbes and depends on both chemical recalcitrance and physical protection from microbes.

2.3.2 Biological pools of carbon in rice soils

The MBC is a good indicator of soil quality and an understanding of the microbial biomass dynamics in rice paddies is essential for managing their nutrient and C cycling (Boddy *et al*., 2007). Banerjee *et al*. (2006) conducted a field experiment to study the SOC and MBC dynamics in the rice-wheat systems and found that use of organic amendments and puddling of soil before rice transplanting increased SOC and MBC contents and MBC showed a seasonal pattern. Lu *et al*. (2002) determine whether the seasonal dynamics of MBC was related to the release of organic substance from rice roots. MBC and DOC in soil solutions were measured over a growing period of rice plants in a pot experiment.

The results suggested that MBC dynamics in rice soil are largely controlled by organic substances released from rice roots.

The living and the most active part of SOC is MBC, therefore, has been suggested as a useful and more sensitive measure of a change in SOC status (Powlson *et al*., 1987; Friedel *et al*., 1996). Majumder *et al*. (2008) evaluated the relationship between soil organic C pools and productivity in a 20-old rice-berseem agroecosystem in West Bengal. Of the organic C pool very labile-C, labile -C, oxidizable-C, MBC and mineralizable-C explained higher variability in crop yields. Pool sizes of microbial biomass in rice soils account for only 2–4% of total C that represent an important and most labile fraction of SOM which is turned over very rapidly (Reichardt *et al*., 1997). With a comparatively rapid rate of turnover of 1–2 years, it is possible to detect changes in microbial fraction long before they are detectable in the total organic matter (Jenkinson and Ladd, 1981). The MBC normally comprises 1–3% of the total SOC. However, this percentage, the 'microbial quotient', has been reported to change in a consistent way and to provide a useful indicator of the soil processes (Anderson and Domsch, 1986).

Whitbread *et al*. (2003) reported that the residue and fertilizer management have influence on nutrient balances, SOM dynamics, and crop yields of a flooded rice system in northeast Thailand (1992–1997) and a wheat–forage legume rotation in eastern Australia (1992-1998). Both soils had been subject to at least 18 years of cultivation and had lost up to 90% of the original labile and 85% of the total carbon. Soil carbon concentrations increased significantly only where higher fertilizer rate and rice stubble retention were combined. Increases in soil C levels require sustained periods of balanced fertilization and residue retention. Soil CO_2 is produced as result of most of the soil microorganisms and its measurement gives the sensitive measure of soil microbial activity. Tate (1979) reported that decomposition of carbon substrates under anaerobic conditions of flooded soils is generally slower than upland soil.

2.4.1 Biological pools of nitrogen in agricultural soils

Nitrogen (N) is essential for the growth and activity of plants and soil microorganisms. N dynamics in the soil–plant system are complex. The N mineralization–immobilization turnover, that is the conversion of organic N to NH_4 and the reverse process that are both carried out by soil microorganisms has been considered to have a major influence on the amount of bioavailability of N in soil (Myrold and Bottomley, 2008). Once organic N is formed by soil microorganisms, three processes can occur: i) organic N can be mineralized to NH_4 –N, which is then released in the extracellular soil environment (Jansson and Persson, 1982; Barraclough, 1997); ii) organic N can be released after cell death or microbial stress (Schimel and Bennett, 2004); iii) mineral N can be released after micro/meso-faunal grazing (the microbial loop) (Clarholm, 1994). Dijkstra *et al*. (2009) tested whether priming effects caused by Fremont cottonwood (*Populus fremontii*) and Ponderosa pine (*Pinus ponderosa*) grown in three different soil types increased plant available N and conclude that soil N availability cannot be determined by soil properties alone, but that is strongly influenced by root–soil interactions. Yan *et al*. (2007) assessed the effect of long-term fertilization on labile organic matter fractions. They analyzed the C and N mineralization and C and N contents in soil, particulate organic matter (POM), light fraction organic matter (LFOM), and microbial biomass. Results showed that soils treated with manure alone had the highest MBC and C and N mineralization. A significant correlation was observed between the C content and N content in soil, POM, LFOM, microbial biomass, or the readily mineralized organic matter.

Bio-available N is an important concept in soil fertility and ecosystem functioning (Griffin, 2008). Dissolved organic N (DON) plays an important role in ecological processes such as N leaching, mineralization, and plant uptake (Nasholm *et al*., 2000; Perakis and Hedin, 2002; Schimel and Bennett, 2004). DON is the fraction of the soil organic N present in dissolved form in soil solution (Murphy *et al*., 2000). DON is considered as the sub-pool of potentially EON that exists as a part of SOM-N. The importance of NH_4-N and NO_3-N in crop nutrition

has focused attention on study of mineral N from the centuries, however a pool of soluble forms of organic N (SON or EON) and DON also exist and it is also slowly gaining interest nowadays. Ros *et al.* (2009) estimated the relative impact of methodological, management and environment factors on EON and DON, using a meta-analysis approach based on 127 studies. Results indicated that the EON and DON pools are neither similar in size nor controlled by the same factors. Zhang *et al*. (2007) carried out a laboratory incubation experiment to evaluate the effects of straw amendment, together with the intensity and frequency of wetting and drying, on microbial processes and water repellency. At a regular interval, soil respiration rate (SRR) on drying and wetting (W/D), MBC and MBN, and soil water repellency after the wetting were measured simultaneously. Rice straw amendment had a greater effect on SRR, but smaller influences on SMB and SMB-C and N than (W/D) frequency and drying intensity. Pikul *et al*. (2007) determined the effect of crop rotation and soil management on SOM, components of SOM, and water-stable aggregation (WSA) of soil near the surface. Measurements were made on soil collected from seven sites representing contrasts between alternative and conventional management. Dry aggregate stability, WSA, soil carbon, SOM, fine particulate organic matter, and coarse POM were measured on aggregates from each aggregate group. There was a significant, positive relationship (r = 0.79) between WSA and fPOM/SOM.

2.4.2 Biological pools of nitrogen in rice soils

Nitrogen is the most essential element in determining the yield potential of rice (Cassman *et al*., 1996; Mae, 1997; Mikkelsen, 1987). In North eastern hill ecosystem where rainfall is very high the N recovery is very less because of more leaching (Saha *et al.* 2007). Soil N fertility is the status of a soil with respect to its ability to provide plants with N in the form, amount, and rate needed for optimum growth. The N fertility of a rice soil primarily depends on: (1) total N content in the soil, (2) N transformations in the soil, and (3) various factors that influence N absorption by rice roots (Kundu and Ladha, 1999). The amount of N mineralized

during a rice-growing season commonly ranges from 3 to 8% of the total N present in the soils (Kundu and Ladha, 1995a). Sooksa-nguan *et al*. (2009) focused the research on the system of rice intensification (SRI) practices to investigate soil microbial processes that may be involved in the SRI yield phenomenon. N mineralization, short-term nitrification, N_2 fixation and denitrification potentials were measured and found that increased nitrification rates and changes in dominant ammonia oxidizing bacteria (AOB) populations have the potential to change N dynamics in the SRI system that could lead to improved yields when other factors are not limiting. Witt *et al*. (1998) investigated the effects of crop rotation and management on soil N dynamics, MBC and MBN in relation to rice N uptake and yield. A maize-rice (M-R) rotation was compared with a rice-rice (R-R) double-cropping system over a 2-year period with four cropping seasons. They concluded that N mineralization-immobilization dynamics in lowland rice systems are sensitive to soil aeration as influenced by residue management in the fallow period and crop rotation, and that these factors have agronomically significant effects on rice N uptake and yield. Sano *et al.* (2006) determined the relationship between the amount of organic matter using various extraction methods and the nitrogen mineralization potential in soil was examined with reference to land use (upland and paddy soils) and soil type (non-volcanic and volcanic soils). The content of labile organic N regulated the soil N mineralization pattern. In the upland soils, the source of N mineralization differed between short-term and long-term mineralization. In the paddy soils, the source of N mineralization was similar, regardless of the duration of mineralization.

2.5.1 Biological pools of phosphorus in agricultural soils

Phosphorus (P) dynamics in soil and maintenance of its adequate quantity are important for sustainability. Many soils throughout the world are P-deficient because the free phosphorus concentration (the form available to plants) even in fertile soils is generally not higher than 10 μM even at pH 6.5 where it is most

soluble (Arnou, 1953). P is present in both inorganic and organic forms. Different forms of P exist in different amounts and proportions, depending on soil type and management. They can be transformed under certain conditions (Sharpley, 2000). P fractionation provides an effective approach for investigating soil P availability and P transformation in soil and the likelihood of its transport (Hedley *et al*., 1982a,b; Sui *et al*., 1999). Among the many properties potentially affected by the level of SOM, the quantity of water-soluble P in soils is of fundamental importance with respect to both crop production and environmental protection. Plant P uptake is very sensitive to P concentration in soil solution (Barber 1995); thus, the maintenance of an adequate concentration of P in soil solution is important for crop production. Nwuke *et al.* (2004) evaluated several crop residues as sources of P for corn (*Zea mays* L.) and noted that the effect of organic residues on P availability was variable and depended on soil characteristics as well as on the nature of the organic source. Sharma *et al*. (1980) reported that in the state of Himachal Pradesh where sizeable area is under acidic soils of P availability pose serious problems due to high P fixing capacity. P mineralized from organic sources is an important factor in determining overall P availability in soil (Stewart and Tiessen 1987; Magid *et al.,* 1996). It has been suggested that mineralization of organic P is mainly through biochemical pathways (hydrolysis by phosphatase), independent of C mineralization, and is controlled by demand for P (Sinsabaugh, 1994); inorganic P can also be released as a by-product from organic matter by biological mineralization and driven by the demand for energy (Stewart and Tiessen, 1987).

Soil P transformations are mediated by microbial activity. Plants not only take up P from the soil but also exert significant effects on soil P availability and dynamics through litterfall, root turnover and exudation, and specific interactions with microbes in the rhizosphere (Attiwill and Adams, 1993; Magid *et al*., 1996). Song *et al*. (2007) conducted a 20-year field trial to study the effects of ecological factors and fertilization on phosphorus characteristics of fertile Udic Mollisols under three ecosystems: (1) bare land ecosystem with no vegetation or fertilizers, (2) natural ecosystem with native grasses but no fertilizers and (3) agro-ecosystem

with a rotation of wheat–soybean–corn. Authors concluded that soil P can be sustained under the natural ecosystem while annual applications of chemical fertilizers and animal manure increased both labile and non-labile P pools in the agro ecosystem.

MBP is considered an important source of P for plant uptake (Macklon *et al.*, 1997) in P-fixing soils (Ayaga *et al*., 2006). Although MBP accounts for only a small fraction (2–5%) of total soil P, the flux of P through microbial biomass can be large, because most P_o compounds in microbial cells (e.g., nucleic acids, phospholipids, and phosphate monoesters) are readily hydrolysable (Oberson *et al.*, 2001; Kouno *et al*., 2002). Microbial biomass as a relatively labile fraction of organic matter is a key site for mineralization of P_o in soils, and thus, is a central feature in the P cycle (Brookes *et al*., 1984). Chen *et al.* (2004) conducted a short-term (40 weeks) glasshouse experiment with 15 grassland soils from around New Zealand to examine the impacts of ryegrass and radiata pine on soil microbial properties and microbiological processes involved in P dynamics. Results showed that the effect of plant species on soil microbial parameters varied greatly with soil type.

Boschetti *et al*. (2009) reported that the P_o was calculated as the difference between total P and P_i. Even though P_o constituted a high percentage of total P, it was not an available P source in these soils. The P_o fractions did not contribute to available soil P and acted as a sink rather than a source of available P during the crop growing cycle.

2.5.2 Biological pools of phosphorus in rice soils

Although soil MBC and N levels are reported routinely, fewer studies provide estimates of MBP. P is an important plant nutrient needed for realizing optimum yields in the Rice-Wheat system (Yadvinder-Singh *et al*., 2000). The supply of P from soil to plants depends on the conversion of organic forms into inorganic forms. Soil microorganisms are key regulators of the biogeochemical P cycle. The availability of P through mineralization can be estimated by

phosphatase activity (Tabatabai, 1994) and MBP in soil. MBP is considered an important source of P for plant uptake (Macklon *et al*., 1997) in P-fixing soils (Ayaga *et al*., 2006). P mineralization is controlled, in part, by the availability of phosphatase enzymes that soil microorganisms and plant roots produce (Tabatabai, 1994). Quantification of phosphorus (P) concentrations in microbial biomass is required to better understand how P immobilization and turnover in soils are controlled by environmental and anthropogenic factors (He *et al*., 2003). In most lowland rice soils, P availability initially increases upon flooding (Ponnamperuma, 1972; Willett, 1986). On clay soils of temperate regions, drainage following soil submergence induced P deficiency in upland crops following rice (Willett *et al*., 1978; Willett, 1979), mainly due to changes among P_i fractions.

2.6 Seasonal dynamics of pools of carbon, nitrogen and phosphorus in soil

Seasonal fluctuations in soil moisture, temperature, and substrate availability can have large effects on microbial biomass and activity. Franzluebbers *et al.* (1994) found that soil MBC changed significantly during the cropping seasons and tillage regimes. The seasonal changes in atmospheric condition also influences number (Diaz-Ravina *et al*., 1993) and mass (Granatstein *et al*., 1987) of soil microbes. MBC fluctuates seasonally in various forest ecosystems (Maithani *et al*., 1996; Bohlen *et al*., 2001; Ruan *et al*., 2004). Feng *et al*. (2009) reported that soil MBC generally decreased from the growing season (July–October) through the dormant season (November–April). The seasonality of soil MBC varies with environmental factors and tree physiology (Myers *et al.,* 2001). In a mixed-oak forest with warm moist summer and cool dry winter, MBC had significant positive correlations with both soil moisture and mean air temperature (Devi and Yadava, 2006). SMB is important for carbon and nutrient cycling in terrestrial ecosystems. Bannerjee *et al*. (2006) suggested that puddling had initial advantage in terms of higher MBC and the non-puddled rice system had a lag phase up to 2 years to build up the microbial biomass. The

assimilation of C into microbial biomass depends on community structure as well as environmental factors such as moisture (Six *et al*., 2006; Feng *et al*., 2003). Lu *et al*. (2002) reported that MBC decreased during the early period of growing rice and then significantly increased during the period of maximum tillering and heading of rice. The decrease in MBC during the early period was likely due to the change in the microbial community structure after soil flooding. Ghoshal and Singh (1995) and Bai *et al.* (2000) observed a substantial decrease in MBC during the early periods and a slight increase during the late periods of rice growth. Ghoshal and Singh (1995) suggested that plant competition for nutrition resources depressed microbial growth during the early periods.

Seasonal shifts in MBC and MBN have been attributed to inputs of mineralizable N (Ross *et al*., 1995) either from plant residues or fertilizer. Sugihara *et al*. (2010) evaluated seasonal variations in MBC and MBN as well as microbial activity (as qCO_2) for 16 months with respect to several factors relating to soil moisture and nutrients under different land management practices in both clayey (38% clay) and sandy (4% clay) croplands in Tanzania and observed that MBC and MBN tended to decreased during the rainy season whereas they tended to increase and remain at high levels during the dry season in all treatment plots. They also found that substantially large seasonal variations in MBC and MBN, continuously high qCO_2, and rapid turnover of soil microbes in sandy soil compared to clayey soil. It was reported that, turnover of nutrients and microbial biomass occurs more rapidly in tropical soils (Balota *et al*., 1998, 2003; Franchini *et al.*,2007) than in temperate regions (Wardle and Ghani, 1995; Wardle *et al*., 1999). Spedding *et al.* (2004) studied the long-term impact of tillage and residue management on soil microorganisms in a sandy loam to loamy sand soil and reported that there is increase in MBN in July followed by an application of 140 kg N ha^{-1} as ammonium nitrate and this application first increased DON. While MBN increased, MBC stayed the same suggesting the occurrence of a shift in soil microbial population in favor of species with elevated N content. It has been shown that levels of MBP in soil are more seasonally variable compared with

MBC (Tate *et al*., 1991), and that microbial biomass is a major source of P in soil solution (Seeling and Jungk, 1996).

Gelsomino *et al*. (2006) investigated the impact of solarization, either with or without addition of farmyard manure, on dynamics of various C, N and P pools in soils. They observed that soil solarization selectively affected the different fractions of total SOM. Total pools of soil C, N and P were left unchanged by the solarization, whereas various labile pools of the native SOM, e.g. the microbial biomass, were readily mineralized, thus rendering larger the available fraction of some soil mineral nutrients, namely N and P forms.

Soil respiration is dependent upon both soil moisture as well as temperature (Buyanovsky and Wagner, 1987). Laik *et al*. (2009) reported that maximum evolution of CO_2 was observed in the month of June followed by September and the lowest was observed in December. The highest soil respiration in the month of June may be attributed to high root respiration, rainfall and temperature. Soil warming, especially during the short summer, can enhance the soil microbial activities and root growth sharply, which leads to an active decomposition of soil carbon matter and the enhancement of plant-derived CO_2 release from root respiration, and results in a quick increase in the soil CO_2 efflux rate (Zheng *et al*., 2009).

The seasonal dynamics of DOC differed significantly between rice planted soils and unplanted soils (Lu *et al*., 2002). DOC in the planted soil showed a rapid increase between the maximum tillering and heading stage of rice and a gradual decrease toward the end of growing season. In unplanted soils, however, DOC concentrations did not show significant fluctuations. The mean DOC concentrations were 3 times higher in planted soils than in unplanted soils. Laik *et al*. (2009) also reported that among the different seasons, the highest concentration of DOC was found during autumn followed by spring and the lowest concentration during winter in the soil layers 0-15 cm and 15-30cm and the upper 15 cm soil layer had more DOC concentration than that of lower layer. The increase in DOC with plant growth illustrated the increase in C substrates, which were readily available for microorganisms.

Soil texture is also an important factor that controls soil microbial dynamics (VanVeen *et al*., 1984; Ladd *et al*., 1992; Muller and Hoper, 2004). Sandy soils are normally characterized by lower amount of SOM, and clayey soil has the high-clay content, protecting soil microbes from predators (Juma, 1993) and dry stress (Van Gestel *et al*., 1991), so that soil microbial biomass is generally lower in sandy soil as compared to clayey soil (Franzluebbers *et al*., 1996). On the other hand, many studies have shown a faster turnover rate of soil microbes in sandy soil as compared to clayey soil (Saggar *et al*., 1999; Muller and Hoper, 2004). Adverse changes in soil properties can also result in permanent reduction in soil productivity and microbial biomass (Srivastava *et al.*, 1989).

2.7 Interaction among carbon, nitrogen and phosphorus pools

Soil is the central organizer of the terrestrial ecosystem. Minerals, organic components, and microorganisms, are the three major components of the soil which are considered as the united system constantly in close association and interacting with each other. Interactions among these soil components have enormous impacts on physical, chemical and biological properties of soil. Interactions of soil minerals with microorganisms and organic components have important role in influencing the stability and degradation of SOM and its associated nutrients (Guggenburger and Haider, 2002) thus, directly affecting the global cycling of C, N and P. Soil microbiota are central to C and N transformations in soils. Cookson *et al*. (2008) conducted the experiment in semi-arid agricultural region of Western Australia on effect of different tillage systems on SOM components, rates of carbon and nitrogen cycling, substrate utilization and microbial community composition. Results indicate that tillage-induced changes to soil pH, and light fraction organic matter, dissolve organic matter (DOM) and microbial biomass pools are likely to be important regulators of the rates of C and N cycling, substrate utilization and microbial community composition in the coarse textured soil. Jones *et al.* (2004) and Cookson *et al*. (2005) reported that DOM is mobile within the soil solution and is thus

considered to have a major role in the transport and supply of C and N to microbial populations. The production and composition of DOM is largely dependent on its equilibrium with total SOM (Gregorich *et al.,* 2000). Therefore, although greater crop residue incorporation might immediately increase total SOM and DOM availability (Gregorich *et al*., 2000), tillage practices which eventually reduce SOM components and are also likely to decrease DOM (Linn and Doran, 1984b). Carbon and nitrogen mineralization are the main processes regulating the availability of nutrients for plants and the release of toxic compounds.

The effect of the interaction between low temperature and soil nutrient status on nutrient transformation rates has been little studied. Net N mineralization has commonly been assumed to decline strongly at low temperatures (Hansen *et al*., 1991). Although soil microbial biomass usually comprises only about 1–3% of total soil organic carbon, it has been recognized as the driving force for mineralization of residues in soil (Abaye and Brookes, 2006). The microbial population size and composition control decomposition dynamics because they are the main source of enzymes in soil (Alef and Nannipieri, 1995). Soil enzymes, which are biological catalyst of specific reactions, play a key role in organic matter decomposition and nutrients transformation and can also be considered good markers of soil biological processes. Manna *et al*. (2005) evaluated the organic matter fractions in soils of 30 years old continuous rice-wheat-jute cropping in West Bengal to examine relationship with yields, nutrient supply capacity and health of soils. The authors observed direct relationship between particulate organic matter carbon or nitrogen (POMC or POMN) and highlighted that the organic pools of C and C–associated nutrients particularly N are good indicators of nutrient supplying capacity of soils and responsible for sustainability of soil quality and productivity.

In mineral wetland soils, N mineralization is maximal at intermediate levels of soil moisture (57–78% waterfilled pore space) (Sleutel *et al*., 2008); the range of moistures producing maximal rates varied with soil texture and SOM. Banerjee *et al*. (2006) and several other researchers (Jenkinson and Ladd, 1981;

Leita *et al.*, 1999) previously reported that there is a significant linear relationship between MBC and SOC in temperate environments. Their results also showed that the similar relationship exists in tropical environments as well where the SOC levels are very low. The change in MBC with SOC depends on crop type and cropping sequence. Such relationships are, however, likely to be affected by several other factors such as soil type, temperature and pH of the soil. Again Rasmussen *et al*. (1998) reported that the N mineralization increased with total soil N content of SOM. Soil microbial biomass itself is an important pool of readily mineralizable organic N in soils (Bonde *et al*., 1988) and is therefore a critical component for assessing potentially mineralizable N (Burket and Dick 1998). Temporal variations in net N mineralization may be linked to changes in MBN since microbial N released after death is readily mineralized by the surviving microorganisms (Lethbridge and Davidson 1983). But Zeller *et al*. (2000) reported that N mineralization is not correlated with MBN.

Organic forms of N and P were important components of the labile pool. Laxminarayana and Patiram (2004) conducted research in some of the rice soils of Mizoram in order to established the relationship between the potentially mineralizable N (pMN) and distribution of inorganic N fractions with organic carbon and found that organic carbon content can be used for predicting available N in the fertilizer adjustments. Decomposition of organic residues and turnover of microbial C and N has been found to be faster in coarse than fine textured soil, as microbes and organic matter are more protected in fine than coarse textured soils (Merckx *et al*., 1985; van Veen *et al*., 1985; Ladd *et al.*, 1992). Aciego Pietri and Brookes (2009) carried a test by soil incubation experiment using wheat straw as substrate and soils of different pHs and the results showed that soil pH has marked effects on microbial biomass, community structure, and response to substrate addition. Van Meeteren *et al*. (2007) evaluate the effect of climate change on ecosystem functioning, the temperature and moisture response of microbial C, N, and P transformations during decomposition of *Calluna vulgaris* litter in a laboratory incubation experiment. The respiration rate, qCO_2, P immobilization rate, net P and N mineralization rate, and nitrification rate

increased with temperature and moisture, while the C and N immobilization rate decreased with increasing temperature and increased with moisture. The temperature sensitivity of Rs (Soil respiration i.e. the Q_{10} value), which refers to the factor by which soil CO_2 efflux increases with an increase in temperature of 10^0C, is an important ecological parameter in ecosystem carbon cycle models (Reichstein *et al*., 2005b). Many field experiments, however, show that Q_{10} values vary spatially (Xu and Qi, 2001b; Lenton and Huntingford, 2003). There was a strong positive correlation between Q_{10} and SOC content at a depth of 20 cm, accounting for 44% of the spatial variation of Q_{10} ($P < 0.001$, Zheng *et al*., 2009). Moreover, a strong dependence of Q_{10} on soil moisture has been quantified in many studies (Xu and Qi, 2001b; Reichstein *et al*., 2002; Janssens and Pilegaard, 2003; Gaumont-Guay *et al*., 2006).

Wang *et al*. (2008) examined the short-term effects of N and P fertilizer application on soil microbial properties, DOM and enzyme activity in a young *Eucalyptus dunnii* plantation at Huitong county, southern China. N application significantly increased soil MBN, mineralized N, DON, and invertase, urease and acid phosphatase activities, but decreased MBC and P, BAS, metabolic quotient and dissolved organic P in comparison with the control. P application decreased MBN, mineralized N, urease and acid phosphatase activities, whereas it increased dissolved organic P, MBP and metabolic quotient.

The altitude of the place also affects the soil properties and nutrient content. Avasthe and Avasthe (1995) studied the effect of altitude on soil properties in relation to available N, P and K of cultivated soils of Sikkim and found that altitude had significant effect on available N and K content of soil. Available N had a significant positive correlation with organic C, available P had a significant correlation with pH, exchangeable Ca and Mg and available K had negative correlation with EC and a positive correlation with silt and clay. Saini *et al*. (2004) reported that higher content microbial biomass C and N in soil leads to increase uptake of N, P and other nutrients by Sorghum, *Sorghum bicolor* L. and Chickpea, *Cicer arietinum* L. A relationship between microbial biomass-C

content and uptake of N and P by plants indicated the importance of soil microbial origin C pool in relation to nutrients availability in soils and uptake by plants.

Anderson and Domsch (1986) and Insam *et al.* (1989) proposed that the ratio of microbial biomass carbon-to-total organic carbon in a soil may serve as a quantitative indicator of carbon loss or accumulation. Microbial biomass also plays a central role in soil nutrient cycling. Strong positive correlations have been found between the amount of nutrients held in the microbial biomass and amounts of mineralizable nutrients in the soil (Carter and MacLeod, 1987; Dalal and Mayer, 1987; Smith, 1993) indicating that nutrient cycling is tightly linked to the turnover of microbial biomass. Substrate quality is one of the most important factors influencing the degradation of plant residues and the activity and size of soil microbial biomass (Paul and Clark, 1996). Moisture is one of the principal environmental factors that determine the rate of organic residue decomposition by microorganisms (Pal and Broadbent, 1975). Decomposition is of crucial importance in biogeochemical cycling and ecosystem functioning. It is known that organic materials decompose less rapidly under anaerobic conditions (Neue and Bloom, 1989).

2.8 Enzyme activity

The parameters related to the biochemical and microbiological states of the soil, are the indicators of the soil microbial activity, principally different enzymatic activities, both specifically related to the cycles of N, P and C (protease, phosphatase and β-glucosidase, respectively) and of a more general nature, such as dehydrogenase, as well as other parameters, such as ATP and respiration. Dehydrogenase activity (DHA), ATP and BAS were significantly affected by season. DHA underwent an increase in summer relative to the other seasons (Bastida *et al.,* 2007). DHA is an enzymatic complex of an intracellular nature (Nannipieri *et al.*, 1990). It participates in the transfer of electrons (Nannipieri *et al.*, 1990) during the oxidative processes involved in energy generation. It shows a positive correlation with BAS (Garcia *et al.*, 1997). But

protease, β-glucosidase and phosphatase are enzymes that act extracellularly, bringing about hydrolysis reactions involving organic compounds in order to produce inorganic compounds. They strongly influence both degradative processes in the soil and changes in organic matter (Ceccanti and Garcı´a, 1994). The role of soil enzyme activities as sensitive indicators of management-induced changes in soil fertility and stress has been widely suggested (Dick *et al*., 1988ab; Masciandaro *et al*., 1998; Nannipieri, 1994). P mineralized from organic sources is an important factor in determining overall P availability in soil (Stewart and Tiessen 1987; Magid *et al*., 1996). Additions of inorganic fertilizer increased Phosphatase activity (Vlasyuk and Lisovan, 1964). Organic matter often leads to an increase in the activity of various enzymes (Crecchio *et al*., 2001; Bhattacharyya *et al*., 2005). Verstraete and Voets (1977) showed that applications of animal manure plus green manure increased phosphatase, urease, saccharase and β-glucosidase activities over a 7-year period and that enzyme activity was related to SOM. DHA increased soon after flooding with the maximum activity being recorded at the grain filling stage and declined sharply at maturity (Nayak *et al*., 2007). Throughout the cropping period, the DHA was higher in treatments receiving compost and was highest in plots receiving both organic and inorganic fertilizers. Soil DHA exhibited a strong negative relationship with Eh and a positive relationship with Fe^{2+} content, suggesting aeration status is the major factor determining the activity (Wlodarczyk *et al*., 2002). It is also known that acid-phosphatase and dehydrogenase activities were higher in soils with P application than that in without P application (Zhong *et al*., 2007).

Review of above cited literatures indicated that depending on land use and covers agro-ecosystems are very different in terms of biological pools of C, N and P as reported from several parts of the world. However, very limited study had been conducted considering C, N and P together for understanding interactions between biological pools of these elements. Though rice ecosystems are one of the major contributors to food grain production, information on biological pools of C, N, and P and interactions among various fractions of these pools and seasonal dynamics are not yet studies in details especially under hill agriculture.

CHAPTER 3

Materials and Methods

3.1 Description of the sampling site

Meghalaya is predominantly hilly and geographically known as "Meghalaya Plateau". Soil sampling was done in two villages viz. Saiden and Kyrdemkulai located in the Ri-Bhoi district of Meghalaya. The elevation of Saiden village is 533 m and Kyrdemkulai village is 808 above mean sea level. Both elevations represent the lower to middle hill elevation range of the state.

3.2 Climate and Soil

Ri-Bhoi district experiences different types of climate ranging from tropical climate in the areas bordering Assam to the temperate climate adjoining the East Khasi Hills District. The areas bordering Assam experience hot-humid weather during summer seasons with average temperature of 30°C especially during the month of May to July and in other areas like Lum Raitong and Lum Sohpetbneng Plateaus, the climate is severely cold during the winter months and is pleasant during the summer period. The usual range of temperature is 25.3^0C maximum and 23.6° minimum. The normal annual rainfall recorded during the year 2000-2010 is 760.0 mm.

Soil of Ri-Bhoi district is classified into hill and plain soils. Patches of red loamy soil and lime silt constitute the major portion. The soils adjoining Assam also consist of heavy loam while in other areas some stones and chips also present. The geological structure of Ri-Bhoi district is composed of Archaean Gneissic complex and Granite is one of the major mineral of this district which has the economic value and will be marketable if properly processed.

3.3 Soil sampling and processing

Rice is the main crop of Ri-bhoi district. It occupies an area of 11,364 ha out of 26,921 ha net sown area. The rice fields of Saiden and Kyrdemkulai villages are selected as study area. The rationale behind selection of these two villages is that each village represents one mega-environment - the Kyrdemkulai site and the valley areas of Manipur state considered one mega-environment, and the Saiden site and Kolashib district of Mizoram constitute another mega-environment. Based on the GGE biplot analysis of multi-locational data of rice varietal trials, these mega-environments were reported (Tripathy *et al.,* 2007). Another reason for selection of these two villages was that rice is usually cultivated in three distinct ecosystems namely hill slopes, transplanted upland terrace and narrow lowland strips between two hills. Out of three rice ecosystems, six soil sampling sites were selected. These sites were: Saiden slope land, Saiden upland terrace-1, Saiden upland terrace-2, Saiden lowland, Kyrdemkulai lowland and Kyrdemkulai upland terrace. Characteristics of these sampling sites are presented in Table 3.1. The soil samples were collected in post-summer time (September 05, 2009) and post-winter time (February 05, 2010) from the above sites. All six sampling sites were occupied by rice crop at the time of collection of soil samples in post-summer. Whereas, rice residues were present in soils at the time of sampling in post-winter except the Saiden low land, where transplanted chilli crop was present in raised beds.

Within each sampling site, 5 random rice plots were selected and from each rice plot 4 random soil samples were collected at 0-15 cm depth. Approximately 1.0 kg of soil sample is collected from each sampling spot and immediately placed on ice box and carried to the laboratory within 8 h of collection. One half of each soil sample was immediately stored at 2˚C until analysis for microbiological parameters and other half was kept open for air drying at laboratory.

Table 3.1 Agricultural management practices at soil sampling sites

Sites	Management of above ground biomass	Use history of inputs	Crops grown
Saiden slope land	Burning of above ground weed biomass practiced every year.	Fertilizers and pesticides never applied.	Traditional rice and occasionally turmeric
Saiden upland terrace-1	Newly created terrace (1 year old). No biomass observed.	DAP was applied @ 30 kg ha^{-1}	Rice monocrop
Saiden upland terrace-2	Weed biomass incorporated into soil at the time of ploughing. 20 years old terrace.	Fertilizers and pesticides never applied	Rice-vegetables
Saiden low land	-do- (Valley between two hills)	Compost and fertilizers applied occasionally (amounts not defined)	Rice – vegetables
Kyrdemkulai low land	-do- (Valley between two hills)	Fertilizers and pesticides never applied	Only rice
Kyrdemkulai upland terrace	-do- (7 years old terrace)	Compost and fertilizers applied to vegetables only (as per recommended practice)	Rice and vegetables

3.4 Soil Analysis

3.4.1 Soil Moisture Content

Soil moisture content was determined gravimetrically by oven drying the soil samples at 105^0C to constant weight (wt.). The moisture content was expressed in percentage (%).

Soil moisture content (%) = {(Fresh soil wt. – dry soil wt.) / dry soil wt.} x 100

3.4.2 pH

Soil samples were analyzed for pH (1:2.5 soil/water suspension) using a standard pH meter (Mettler Toledo, Switzerland).

3.4.3 Total Organic Carbon (TOC)

Air dry soil sample was grinded in pestle and mortar, and 0.5 g of finely grinded soil was used to determine TOC by following wet oxidation method described by Walkley and Black (1947). Soil sample (0.5 g) was mixed with 10 ml of 1N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 in a 500 ml capacity conical flask and left for 30 min. After oxidation, 200 ml distilled water was added to the conical flask. 5 ml H_3PO_4 and $AgSO_4$ (5 g $AgSO_4$ added per litre basis) was added to the flask. The residual $K_2Cr_2O_7$ was titrated with freshly prepared 0.5 M ammonium ferrous sulfate in presence of diphenylamine indicator using a digital burette (Eppendorf Make, Germany). TOC content in soil was expressed in g $100g^{-1}$ soil (%). Inorganic carbon fractions in soils were negligible and hence, TOC content was used to determine C:N ratio in the soils.

3.4.4 Dissolved Organic Carbon (DOC)

Extraction of DOC in field moist soil samples was done by using 1M KCl solution at a ratio of 5:1(v/w) in an overhead shaker for 30 mins. Soil suspension

was filtered through 0.45 µm Whatmann nylon membranes (Zsolnay, 1996; McDowell *et al*., 2006) and an aliquot (10 ml) of filtered solution was used for determination of C content by wet oxidation method as described for TOC in the previous section 3.3.4. DOC content is express in µg g^{-1}(dw) soil.

3.4.5 Total Nitrogen (TN)

Air dried finely grinded soil sample (1 g) was used for determination of TN by regular Kjeldahl method as described by Bremner and Mulvaney (1970). In brief, 1 g soil sample was taken in a digestion tube and to this 10 ml distilled water was added to moisture the sampl. Approximate 2 g of digestion mixture (Composition: 100 part of K_2SO_4 (100 g), 10 parts of $CuSO_4.5H_2O$ (10 g) and 1 part of Selenium powder (1g) mixed together and grinded it to get fine powder) and 10 ml of conc. H_2SO_4 were added and then suspension was digested in an aluminium heating block (KEL Plus, Pelican Equipment, Chennai) at 360 °C for 2 h. After completion of digestion (the end point was thick clear copper coloured soup at the bottom of the digestion tube), 40 ml each of 0.32% $KMnO_4$ and 40% NaOH and 5 ml of paraffin wax (heavy liquid) were added to the tube and distillation was carried out for 6 min using a automatic distillation chamber (Classic DX, Pelican Equipment, Chennai). Ammonia generated during distillation was collected in 20 ml of 2% boric acid solution containing few drops of mixed indicator (Composition of mixed indicator: 0.1 g bromocresol green with 0.07 g of Methyl red and dissolved this mixture in 100 ml ethanol). Finally, amount of boric acid used for absorption of ammonia was determined by titrating with a 0.1 N standard H_2SO_4 using a digital burette (Eppendorf Make, Germany). TN was expressed in kg ha^{-1}.

3.4.6 Extractable Organic Nitrogen (EON)

Extractable portion of total nitrogen was obtained by shaking field moist soil with 0.5 M K_2SO_4 (soil and salt solution at 1:4) for 30 min at 200 rpm (Ros *et*

al., 2009). Soil suspension was filtered through Whatmann no. 42 and a small volume (10 ml) of the supernatant was taken for determination of total extractable N content by the regular Kjeldahl method (Bremner, 1970) similar to that described for TN in the section 3.3.5. Inorganic portion of N (NH_4^+ -N and NO_3^- -N) in the filtered supernatant was also determined by the MgO-Devarda alloy method after destruction of NO_2^- with sulfamic acid (Keeney and Nelson, 1982). Then, extractable organic N was estimated as the difference between extractable total N and extractable inorganic N. EON was expressed in μg g^{-1} (dw) soil.

3.4.7 Soil Available Nitrogen (Avl-N)

An amount (5 g) of finely grinded soil sample was used for determination of Avl-N by alkaline permanganate oxidation method described by Subbiah and Asija (1956). Soil sample was taken in a distillation tube and to this 40 ml of 0.32% $KMnO_4$, 40 ml of 2.5% NaOH and 5 ml of paraffin wax (heavy liquid) were added and distilled for 4 min in an automated distillation chamber (Classic DX, Pelican Equipment, Chennai). Ammonia generated during distillation was collected in 2% boric acid containing few drops of mixed indicator (Composition: of mixed indicator: 0.1 g bromocresol green with 0.07 g of Methyl red and dissolved this mixture in 100 ml ethanol) in a conical flask and finally amount of boric acid used for absorption of ammonia was determined by titrating with standard 0.1N H_2SO_4 using a digital burette (Eppendorf Make, Germany). Avl. N was expressed in kg ha^{-1}.

3.4.8 Total Phosphorus (TP)

Total P was determined by digesting the soil sample (0.5 g air dried finely grinded) with 10 ml of diacid mixture ($HNO_3/HClO_4$, 3:1) at 200^0C for 2.5 h in a digestion block (Kjel Plus, Pelican Equipment, Chennai) until the mixture turns white, as described by (Olsen and Sommers, 1982). The digested mixture was

diluted with double distilled water and filtered through Whatman no. 42 in 100 ml volumetric flask and final volume made up to 100 ml. A portion of the filtrate (10 ml) was taken in a 50 ml volumetric flask and to this 10 ml of Ammonium paramolybdate-vanadate reagent and then final volume was made up with double distilled water. The stable yellow colour in sample was determined using a spectrophorometer (Spectrascan UV-2600, Thermo Scientific, USA) at 480 nm and concentration of P was read from the standard curve. TP was expressed as kg ha^{-1}.

3.4.9 Soil Available Phosphorus (Avl-P)

Bray's P in soil was determined by following the stannous chloride blue colour method (Bray and Kurtz, 1945; Page *et a.,* 1982). Soil sample (5 g of air dried finely grinded) was extracted with 50 ml of 0.03N NH_4F in 0.025N HCl for 5 min in a reciprocating shaker. Soil suspension was filtered through Whatmann No. 42 and 5 ml of the supernatant was used for developing blue colour using Dickman Bray's reagent and stannous chloride. Finally, intensity of blue colour was measured at 660 nm (Spectrascan UV-2600, Thermo Scientific, USA) and concentration of P was read from the standard curve. Avl-P was expressed as kg ha^{-1}.

3.5 Soil Biochemical Analysis

3.5.1 Microbial Biomass Carbon (MBC)

Freshly collected soil samples were used for MBC determination by the procedure chloroform-fumigation-extraction method (Brookes and Joergensen, 2006). Root debris or visible organic residues were removed from the sample. Each soil sample was divided into two sub samples (each sub sample weight was 25 g). Each sub sample was taken in 50 ml beaker. One sub-sample was fumigated for 24 h with chloroform vapour in a desiccator and the other sub-sample kept in a dessicator as check without fumigation for 24 h. After 24 h

incubation, the residual chloroform in the fumigated sample was removed by evacuation. To each sub-sample 100 ml 0.5 M K_2SO_4 (1:4 ratio) was added and shaked the sample for 30 min at 200 rpm in an orbital shaker (Rotek LES, Pelican Equipment, Channai). Then, soil suspensions were filtered through a Whatmann No. 42 filter paper. 10 ml of the supernatants were used for determination of C by wet oxidation method similar to that described for determination to TOC in the section 3.4.3. The difference in C content between fumigated and non-fumigated sub-samples was determined and then, MBC was calculated using a conversion factor, K_{EC} = 0.38 (Vance *et al*., 1987; Wu *et al*., 1990; Dilly and Munch, 1998). MBC content was expressed in µg g^{-1} (dw) soil.

3.5.2 Microbial Biomass Nitrogen (MBN)

MBN was determined in freshly collected soil sample using the chloroform-fumigation-extraction method (Brookes and Joergensen, 2006). The supernatants obtained after fumigation and extraction steps in the determination of MBC were also used for determination of N content. Regular Kjeldahl method was used to determine N content in 10 ml portion of the supernatant of each of the fumigated and non-fumigated samples by following the similar procedure used for TN determination in the section 3.4.5. The difference in N content between fumigated and non-fumigated sub-samples was determined and then, MBN was calculated by using the conversion factor, K_{EN} = 0.45 (Gijsman *et al*., 1997; Jenkinson, 1988; Ross and Tate, 1993). MBN was expressed as µg g^{-1} (dw) soil.

3.5.3 Microbial Biomass Phosphorus (MBP)

MBP was determined in freshly collected soil sample using the chloroform-fumigation-extraction method (Brookes and Joergensen, 2006). The supernatants obtained after fumigation and extraction steps in the determination of MBC were also used for determination of inorganic P content. Bray's I method was used to determine inorganic P content in 10 ml portion of the supernatant of

each of the fumigated and non-fumigated samples by following the similar procedure used for Avl-P determination in the section 3.4.9. The difference in inorganic P content between fumigated and non-fumigated sub-samples was determined and then, MBP was calculated by using the conversion factor, K_{Ep} = 0.40 (Brookes *et al*., 1982; Gijsman *et al*., 1997). MBP was expressed as µg g^{-1} (dw) soil.

3.5.4 Potentially Mineralizable Nitrogen (pMN)

Static anaerobic incubation procedure was used to determine pMN infreshly collected soil samples. Each soil sample was divided in to two sub-samples. From one sub-sample, 16 g portion of soil was taken in test tube (2.8 cm diameter and 60 ml capacity) and flooded the soil by adding 40 ml distilled water and. Trapped air was removed by lightly tapping the tubes and then, closed the mouth by rubber stopper. Soils were incubated at 40˚C for 7 days. Other sub-sample that was not to be incubated was also processed similar way. The inorganic NH_4-N produced during 7 days incubation under anaerobic situation was extracted using 40 ml of 4M KCl solution by shaking 1 h and finally filtered through Whatman No. 42 filter paper. Mineralized N during 7 days incubation was calculated by subtracting the NH_4-N measured in the sample that was not incubated from that measured in the incubated sample (Page *et al*., 1982; Franzleub-bers *et al*., 2000; Tirol- Padre *et al*., 2007). pMN is expressed in µg g^{-1} (dw) soil.

3.5.5 Basal Respiration (BAS)

BAS was measured in fresh soil samples by using standard base trap method in a NaOH solution (Zibilske, 1994; Pell *et al*., 2006; Ohlinger *et al*., 1996). Soil sample (40 g) at water holding capacity of 50% was weighed and put in plastic cup and then, the cup was placed inside an incubation vessel. A scintillation vial containing 2 ml of 1N NaOH was placed inside the vessel and

the, mouth of the vessel was made air tight. Finally, vessel was incubated for 10 days at 22^0C constant temperature. After 10 days of incubation the scintillation vial was replaced with a new scintillation vial containing 2 ml of freshly prepared 0.1 N NaOH and incubation advanced for 24 h at 22^0C constant temperature. After completion of incubation scintillation vial was taken out and 4 ml 0.05M $BaCl_2$ solution was added to the NaOH and the excess of hydroxide was titrate with 0.05M HCl in the presence of phenolphthalein indicator. BAS was expressed as μg CO_2 g^{-1} (dw) soil h^{-1}.

3.5.6 Substrate Induced Respiration (SIR)

After establishment of BAS, SIR was determined in the same soil sample in the incubation vessel by addition of glucose (Alvarez and Alvarez, 2000; Anderson and Domsch, 1978). Soil and glucose are mixed thoroughly with spatula and 3ml of 1M NaOH in scintillation vial was placed inside the incubation vessel and then, incubated at 22 ^{0}C for 21 days. The excess of NaOH was titrated against 0.05M HCl at 7, 14 and 21 days and the set up without soil sample was also maintained as check and CO_2 evolution was calculated per hour basis. SIR was expressed as μg C g^{-1} (dw) soil h^{-1}.

3.5.7 Dehydrogenase Activity (DHA)

DHA was determined in air dried soil samples as per the method described by Casida *et al*. (1964). Soil sample (10 g) was mixed with 0.1g $CaCO_3$ and then, the mixture was distributed to three screw cap test tubes each with 3 g. To each test tube 0.5ml of 3% 2,3,5-triphenly-tetrazolium chloride (TTC) and 1.25ml of distilled water were added and mixed thoroughly by gentle tapering and incubated it at 37°C for 24h. After 24 h incubation, the soil suspension was filtered through glass funnel equipped with absorbent cotton. Methanol was used to extract the soil suspension until the cotton plug's colour became white and the final volume was made up to 50 ml. Intensity of reddish colour was measured by using

spectrophotometer at a wavelength of 485nm (Spectrascan 2600, Thermo Scientific, USA). The concentration of triphenyl formazan (TPF) in the supernatant was determined against a standard graph prepared using known concentrations of TPF. DHA was expressed as µg (TPF) g^{-1} (dw) soil h^{-1}.

3.5.8 Phosphatase Activity (PHA)

PHA was determined in fresh soil samples as per the procedure described by Tabatabai and Bremner (1969). Soil sample (1 g) was taken in a Erlenmeyer flask and to this 4 ml of MUB (Modified Universal Buffer, pH 6.5), 0.25 ml of toluene and 1 ml of p-NPP (p-nitrophenyl phosphate) were added and incubate at 37°C for 1 h. After incubation, 1 ml of 0.5 M $CaCl_2$ and 4 ml 0.5M NaOH were added to the soil suspension and filtered. Intensity of yellow colour was measured in the filtrate at 400 nm using a spectrophotometer (Spectrascan 2600, Thermo Scientific, USA). The concentration of p-nitrophenol in the filtrate was determined against a standard curve prepared by using p-nitrophenol standard solution. PHA was expressed as µg p-nitrophenol g^{-1} (dw) soil h^{-1}.

3.6 Statistical Analysis

3.6.1 Univariate statistics

All univariate analyses were performed using SPSS v12.0 (SPSS Inc., Chicago, IL, USA). Within a site, each parameter analysed for different seasons was normally distributed as determined using Kolmogorov-Smirnov test and any significance difference between seasons was performed by paired t-test ($P<0.05$).

3.6.2 Multivariate statistics

The data matrix (all the analysed parameters as rows and the different sampling sites as columns) was tested for multivariate normality test by

performing simple scatter plots of all pairwise combinations of variables (Draftsman plot analysis). On basis of the multivariate normality check test, the data matrix was square-root transformed and normalized the data matrix to eliminate the effects of different units for different parameters while performing principal component analysis (PCA). All the parameters and sites were ordinated in two dimensions based on the scores of the variables (parameters) in the first two principal components and the euclidean distance was used as a measure of dissimilarity between sites. Dendrogram was constructed by hierarchical cluster analysis (group-average linking) using the Bray–Curtis resemblance matrix (Clarke, 1999). Resulting clusters were superimposed on the PCA plot to form ellipses at arbitrary resemblance levels of slices drawn through the dendrograms. The pair-wise correlation matrix was also developed on the normalised data set to find out the relationship between various parameters irrespective of sites. The PCA, hierarchical cluster, Bray-Curtis resemblance matrix, correlation matrix analyses presented in this thesis was computed using PRIMER-E v6.1.9 software (Primer-E Ltd, Plymouth, UK).

CHAPTER 4

Experimental Findings

Three rice ecosystems (direct-seeded hill slope, transplanted upland terrace, and lowland) were selected from two villages viz. Saiden and Kyrdemkulai of the Ri-Bhoi district of Meghalaya in this investigation for assessment of biological pools of C, N and P. These rice ecosystems were compared in terms of sizes of the biological pools and their seasonal dynamics.

4.1 Soil particle distribution, moisture content, pH and C:N ratio

Soil particle distribution, textural class, moisture content, pH and C:N ratio of soils of six rice fields were presented in Table 4.1. Soils of Saiden upland terrace 1 and 2 and Kyrdemkulai upland terrace were loam, Saiden lowland was clay loam, Kyrdemkulai lowland was silt loam and Saiden slope land was Sandy clay loam. Soil moisture content in rice fields varied widely depending on land use types. Moisture content in soils of the direct-seeded slope land rice fields was the lowest (14-28%) in post summer and post-winter. Soils of lowland rice fields were waterlogged during both seasons except the soil moisture content during post-winter in Saiden lowland field i.e. 62.7%. Moisture content in soils of Saiden upland terrace-2 rice field was optimum (53.2 to 60.0%) in both seasons, whereas the moisture content in soils of Kyrdemkulai upland terrace rice field was lower i.e. 22% in post-winter as against 61.4% during post-summer season.

Soil pH in rice fields varied in the range from a minimum of 4.80 to a maximum of 5.60 (Table 4.1). Soil pH in post-winter was found to be significantly higher (paired t-test, $P<0.01$) as compared to that in post-summer in all rice fields, except the Saiden slope land and Saiden terrace-2, where seasonal

variation was non-significant. Soil pH in rice fields was observed in the textural class order of loam (pH range 5.2 to 5.6) > clay loam to silt loam (pH range 4.92 to 5.1) > sandy loam (pH range 4.82 to 4.85). Soil C:N ratios in rice fields were varied in the range from 7.9:1 to 13.0:1 (Table 4.1). Seasonal fluctuation in soil C:N ratio i.e. a significant higher soil C:N ratio in post-summer compared to that in post-winter was observed in Saiden slope land, Saiden upland terrace-1 and Kyrdemkulai upland terrace; whereas seasonal change in C:N ratios in soils of lowland and Saiden upland terrace-2 was non-significant.

4.2 Soil carbon fractions

Within Kyrdemkulai sampling location, lowland rice soil contained 44% more TOC over that in upland terrace soil. Similarly, within Saiden sampling location, lowland rice soil contained 33%, 55% and 60% more TOC over that in upland terrace-2, slope land and upland terrace-1, respectively (Table 4.2). TOC contents in soils of different rice fields were varied significantly between seasons depending on rice ecosystems. TOC content in soils of lowland rice ecosystems was significantly higher in post-winter as compared to that in post-summer (P<0.01). Whereas, TOC content in soil of Saiden slope land rice field was significantly higher in post-summer as compared to that in post-winter season (P<0.01). No significant seasonal variations in TOC contents in soils of Saiden Upland terrace -1 and terrace-2 and Kyrdemukulai upland terrace rice fields were observed (P>0.05).

DOC content in soils of different rice fields maintained a similar trend like TOC contents (Table 4.2). DOC content was the highest {1398 to 1718 µg g^{-1} (dw) soil} in soils of lowland rice fields followed by {1002 to 1280 µg g^{-1} (dw) soil} in soils of upland terrace rice fields and the lowest {508 to 831 µg g^{-1} (dw) soil} in soils of Saiden slope land rice fields. DOC and moisture content in soils maintained a significant correlation (r = 0.86, n = 30, P<0.01) irrespective of rice ecosystems and seasons. MBC content in soils of rice fields was found to be in the

Table 4.1 Some soil properties of sampling sites under different rice ecosystems

Site	Season	Soil particle distribution (%)			Textural class	MC (%)	pH	Soil C:N
		Sand	Silt	Clay				
Saiden slope land	Post summer	50.0	25.0	25.0	Sandy clay loam	28.1±0.4[a]	4.85±0.05[a]	11.0:1[b]
	Post winter	ND	ND	ND		13.9±0.6[b]	4.80±0.05[a]	9.5:1[a]
Saiden upland terrace-1	Post summer	38.0	40.0	22.0	Loam	32.1±0.8[a]	5.41±0.03[a]	13.0:1[b]
	Post winter	ND	ND	ND		32.0±1.2[a]	5.60±0.08[b]	10.3:1[a]
Saiden upland terrace-2	Post summer	36.5	42.5	21.0	Loam	60.0±4.3[a]	5.27±0.04[a]	7.9:1[a]
	Post winter	ND	ND	ND		53.2±3.7[b]	5.30±0.06[a]	7.9:1[a]
Saiden low land	Post summer	34.5	38.5	27.0	Clay loam	waterlogged	5.04±0.02[a]	9.5:1[a]
	Post winter	ND	ND	ND		62.7±0.9	5.10±0.04[b]	10.2:1[a]
Kyrdemkulai low land	Post summer	26.0	52.0	22.0	Silt loam	waterlogged	4.92±0.06[a]	11.4:1[a]
	Post winter	ND	ND	ND		waterlogged	5.09±0.02[b]	11.8:1[a]
Kyrdemkulai upland terrace	Post summer	40.0	34.5	25.5	Loam	61.4±1.2[a]	5.20±0.04[a]	9.3:1[b]
	Post winter	ND	ND	ND		22.0±0.4[b]	5.30±0.02[b]	8.2:1[a]

[MC - moisture content; C:N – carbon to nitrogen ratio; ND – not determined because within a season soil textural class is hardly changed; within a site for each parameter, values that differ significantly (P<0.01) are followed by different letters, as determined by paired t-test.]

order of slope land rice field < upland terrace rice field < lowland rice field. Except Saiden slope land and Kyrdemkulai upland terrace rice fields, soils of all other rice fields contained significant higher MBC in post-winter than that in post-summer (P<0.01). BAS was found to be significantly higher in post-summer compared to that in post-winter in upland terrace and slope land soils and were comparable between these fields. Overall, higher BAS was observed in soils of lowlands, but seasonal fluctuation of BAS in lowlands was non-significant (P>0.05). Soil moisture showed significant positive correlation with BAS or MBC (r = 0.85 and 0.87, respectively; n=30, P<0.01). Glucose induced soil respiration in the first week of incubation was significantly higher in post-summer as compared to that in post-winter, irrespective of rice fields (Fig. 4.1). Glucose induced soil respiration was found to be the highest in soil of Saiden lowland rice field and the lowest in soil of Saiden slope land rice field.

4.3 Soil nitrogen fractions

TN content was the highest (average 6000 kg N ha^{-1}) in soils of lowland rice fields followed by 5592 kg N ha^{-1} in soils of upland terrace rice fields, 3760 kg N ha^{-1} in slope land rice field and the lowest (3248 kg N ha^{-1}) in soil of Saiden upland terrace-1, irrespective of seasons (Table 4.3). TN content was significantly higher in post-winter as compared to that in post-summer in soils of all rice fields, except the direct-seeded slope rice field, where seasonal variation in TN content was non-significant (P>0.05).

MBN, EON and pMN in soils of different rice fields varied from 64.0 to 206.6, 188 to 454 and 32.9 to 79.8 µg g^{-1} (dw) soil, respectively (Table 4.3). All these parameters were higher in terms of their contents in lowland rice soils. Kyrdemkulai upland terrace and Saiden slope land soils contained significant lower amount of MBN and EON in post-winter in comparison to MBN and EON contents in post-summer (P<0.01). Seasonal variation of pMN was non-significant (P>0.05) in lowland rice fields, Saiden upland terrace-1 and slope land; otherwise, pMN varied significantly between seasons in other rice fields. It

Table 4.2 Organic carbon fractions and basal respiration in soils of rice ecosystems as influenced by seasons

Site	Season	TOC (%)	DOC µg g^{-1} (dw) soil	MBC µg g^{-1} (dw) soil	BAS µg CO_2 g^{-1} (dw) soil h^{-1}
Saiden slope land	Post summer	1.82±0.03[b]	831±4.0[a]	667±11.4[b]	0.35±0.06[b]
	Post winter	1.61±0.02[a]	508±16.0[b]	448±9.7[a]	0.21±0.01[a]
Saiden upland terrace-1	Post summer	1.69±0.04[a]	1002±16.6[b]	671±7.8[a]	0.42±0.03[a]
	Post winter	1.65±0.05[a]	1145±14.2[a]	747±17.3[b]	0.38±0.02[b]
Saiden upland terrace-2	Post summer	1.98±0.06[a]	1123±15.5[b]	708±11.6[a]	0.45±0.04[a]
	Post winter	2.05±0.08[a]	1230±24.0[a]	936±21.4[b]	0.39±0.04[b]
Saiden low land	Post summer	2.48±0.03[a]	1398±15.6[a]	1072±9.9[a]	0.48±0.05[a]
	Post winter	2.87±0.02[b]	1573±32.2[b]	1396±125.3[b]	0.47±0.01[a]
Kyrdemkulai low land	Post summer	2.94±0.07[a]	1718±17.0[b]	1405±21.4[b]	0.43±0.04[a]
	Post winter	3.20±0.04[b]	1557±43.2[a]	1377±26.0[a]	0.44±0.02[a]
Kyrdemkulai upland terrace	Post summer	2.13±0.04[a]	1280±22.2[b]	992±7.0[b]	0.41±0.07[b]
	Post winter	2.12±0.06[a]	1033±14.5[a]	534±37.3[a]	0.25±0.01[a]

[TOC- total organic carbon, DOC – dissolved organic carbon, MBC – microbial biomass carbon, BAS – basal respiration. Within a site for each parameter, values that differ significantly (P<0.01) are followed by different letters, as determined by paired t-test.]

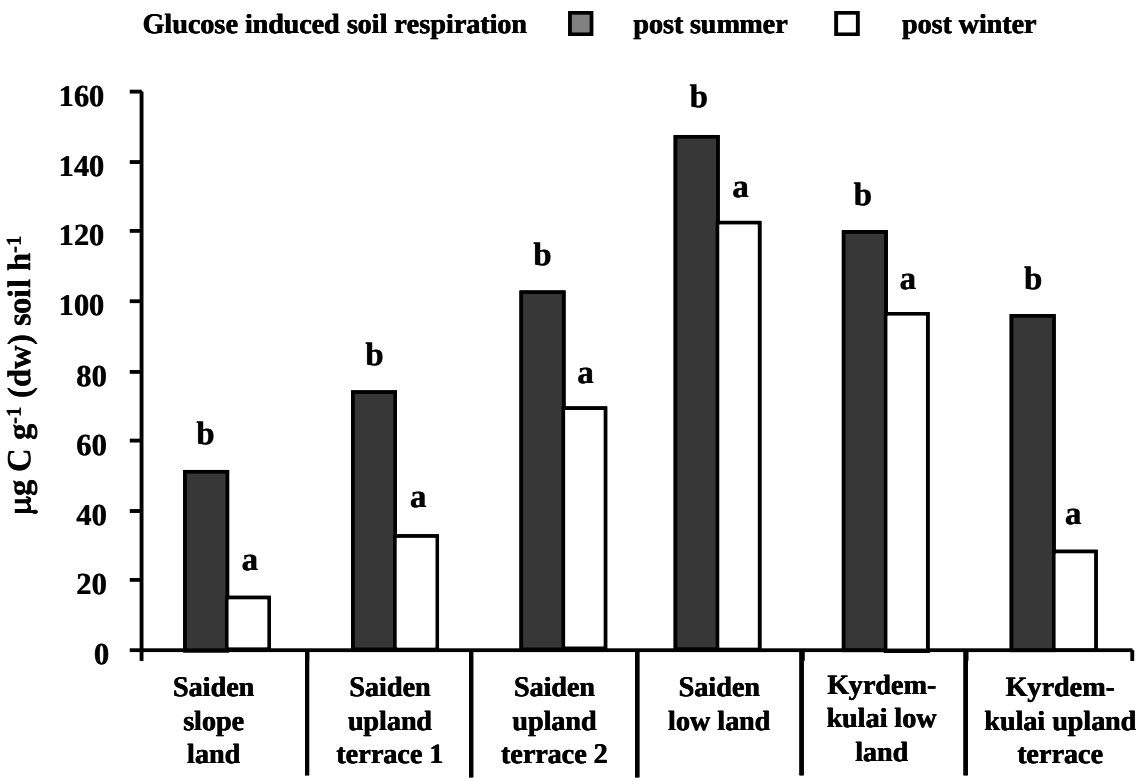

Fig. 4.1 Induced soil respiration due to addition of glucose in soils of different rice ecosystems as influenced by seasons. Within a site, values that differ significantly are followed by different letters at $P \leq 0.01$. Amount of CO_2-C produced inside incubation vessels during 7 days of incubation was trapped in NaOH solution and data used for calculation of glucose induced soil respiration.

was observed that EON and moisture content in soils maintained a significant correlation (r = 0.53, n = 30, $P<0.01$) irrespective of rice fields. Avl-N content was the highest (average 627.5 kg N ha^{-1}) in soils of lowland rice fields followed by 421.0 kg N ha^{-1} in soils of upland terrace rice fields, average 372.5 kg N ha^{-1} in slope land rice field and the lowest (286.5 kg N ha^{-1}) in soil of Saiden upland terrace-1, irrespective of seasons (Table 4.3). Avl-N in soils of lowland did not vary between seasons, however seasonal variation in Avl-N was pronounced in soils of Saiden upland terrace 1 and 2 and Kyrdemkulai upland terrace.

4.4 Soil phosphorous fractions

TP contents in rice soils ranged from 1077 to 1699 kg P ha^{-1} and the contents were in the order of lowland rice fields > upland terrace rice fields > slope land rice field (Table 4.4). TN contents in soils varied between seasons but effect was non significant ($P>0.05$). Avl-P in rice soils ranged between 5.69 to 28.4 kg ha^{-1}. Avl-P content in soils was significantly higher in post-winter seasons in compassion to that in post-summer in all rice fields with the only exception in Saiden slope land. MBP content was the highest {28.2 to 39.8 µg g^{-1} (dw) soil} in soils of lowland rice fields, and all other rice fields were comparable in terms of MBP contents in soils, except the lowest MBP content {9.9 µg g^{-1} (dw) soil} in soils of Kyrdemkulai upland terrace in post-winter (Table 4.4).

4.5 Microbial biomass C, N and P to total C, N and P in soils

Contributions of microbial biomass C, N, and P to the soil total C, N, and P, respectively were found to be higher in lowland rice fields followed by upland terrace rice fields and the lowest contribution was observed in direct-seeded slope rice field including Kyrdemkulai upland terrace rice field in post-winter (Table 4.5). The C:N:P ratios of microbial biomass in soils of rice fields varied in the range from 35.3**:**4.9**:**1 to 61.0**:**6.1**:**1 with an indication of narrow ratio in lowland rice soils and wider ratio in soils of Saiden and Kyrdemkulai upland terraces.

Table 4.3 Nitrogen fractions in soils of rice ecosystems as influenced by seasons

Site	Season	kg ha^{-1}		µg g^{-1} (dw) soil		
		TN	Avl-N	MBN	EON	pMN
Saiden slope land	Post summer	3706±155^{a}	373±17.5^{a}	102.0±9.6^{b}	283±13.4^{a}	32.9±2.1^{b}
	Post winter	3813±146^{a}	372±10.2^{a}	66.8±2.2^{a}	175±10.0^{b}	37.7±3.5^{a}
Saiden upland terrace-1	Post summer	2967±142^{b}	267±14.2^{a}	73.8±7.5^{b}	174±14.9^{b}	33.9±3.3^{a}
	Post winter	3528±144^{a}	306±15.7^{b}	93.4±6.1^{a}	203±18.6^{a}	34.4±3.5^{a}
Saiden upland terrace-2	Post summer	5587±65^{b}	462±8.5^{a}	92.1±12.9^{a}	217±16.2^{a}	41.3±2.1^{b}
	Post winter	5800±219^{a}	554±14.7^{b}	94.0±18.4^{a}	236±13.8^{a}	63.4±4.2^{a}
Saiden low land	Post summer	5829±141^{b}	587±25.9^{a}	161.0±17.1^{b}	217±17.4^{b}	75.8±5.2^{a}
	Post winter	6196±194^{a}	601±27.0^{a}	182.0±14.7^{a}	436±65.9^{a}	79.8±4.6^{a}
Kyrdemkulai low land	Post summer	5905±90^{a}	647±34.0^{a}	197.0±14.8^{a}	283±14.5^{b}	69.4±2.0^{a}
	Post winter	6071±146^{b}	675±28.5^{a}	206.6±19.4^{a}	454±51.5^{a}	68.4±2.7^{a}
Kyrdemkulai upland terrace	Post summer	5263±76^{b}	552±23.0^{a}	148.9±16.3^{a}	278±10.4^{a}	62.8±3.1^{a}
	Post winter	5720±116^{a}	384±16.1^{b}	64.0±5.4^{b}	188±10.6^{b}	52.6±3.2^{b}

[TN – total nitrogen, Avl-N – available nitrogen, MBN – microbial biomass nitrogen, EON – extractable organic nitrogen, pMN – potentially mineralizable nitrogen. Within a site for each parameter, values that differ significantly (P<0.01) are followed by different letters, as determined by paired t-test.]

Table 4.4 Phosphorus fractions in soils of different rice ecosystems as influenced by seasons

Site	Season	TP	Avl-P	MBP
		kg ha^{-1}	kg ha^{-1}	µg g^{-1} (dw) soil
Saiden slope land	Post summer	1077±14[a]	20.1±1.3[a]	18.4±0.5[b]
	Post winter	1120±58[a]	17.5±0.9[b]	11.9±0.4[a]
Saiden upland terrace-1	Post summer	1385±37[a]	10.9±0.6[b]	16.4±0.4[a]
	Post winter	1287±37[b]	15.9±0.7[a]	17.7±0.3[b]
Saiden upland terrace-2	Post summer	1319±30[a]	5.7±0.3[b]	16.2±0.3[a]
	Post winter	1254±73[a]	12.4±0.7[a]	15.4±0.7[a]
Saiden low land	Post summer	1570±96[a]	15.9±0.5[b]	28.2±1.8[a]
	Post winter	1699±92[a]	18.4±0.7[a]	32.1±2.2[b]
Kyrdemkulai low land	Post summer	1436±40[a]	6.2±0.3[b]	39.8±3.6[a]
	Post winter	1398±37[a]	10.5±0.7[a]	36.5±3.5[a]
Kyrdemkulai upland terrace	Post summer	1385±30[a]	12.8±0.5[b]	26.1±2.4[a]
	Post winter	1363±34[a]	19.7±0.7[a]	9.9±0.4[b]

[TP – total phosphorus, Avl-P – available phosphorus, MBP – microbial biomass phosphorus. Within a site for each parameter, values that differ significantly (P<0.01) are followed by different letters, as determined by paired t-test.]

Table 4.5 Contribution of microbial biomass carbon, nitrogen and phosphorus to total soil carbon, nitrogen and phosphorus in soils of different rice ecosystems

Site	Season	MBC (x100) –to- TOC$^{\alpha}$	MBN (x100) –to- TN$^{\beta}$	MBP (x100) – to - TP$^{\gamma}$	C:N:P of microbial biomass
Saiden slope land	Post summer	3.3	6.2	3.8	36.3:5.5:1
	Post winter	2.8	3.9	2.4	37.6:5.6:1
Saiden upland terrace-1	Post summer	4.0	5.6	2.7	40.9:4.5:1
	Post winter	4.5	5.9	3.1	42.2:5.3:1
Saiden upland terrace-2	Post summer	3.7	3.7	2.8	43.7:5.7:1
	Post winter	4.3	3.6	2.7	61.0:6.1:1
Saiden low land	Post summer	4.3	6.2	4.0	38.0:5.7:1
	Post winter	4.9	6.6	4.2	43.5:5.7:1
Kyrdemkulai low land	Post summer	4.8	7.5	6.2	35.3:4.9:1
	Post winter	4.3	7.6	5.8	37.7:5.7:1
Kyrdemkulai upland terrace	Post summer	4.7	6.3	4.2	38.0:5.7:1
	Post winter	2.5	2.5	1.6	54.0:6.5:1

[MBC – microbial biomass carbon, MBN – microbial biomass nitrogen, MBP – microbial biomass phosphorus, TOC – total organic carbon, TN – total nitrogen, TP – total phosphorus. $^{\alpha\ \beta\ \gamma}$ values were derived from the means of the parameters presented in previous tables and hence, no statistics was performed.]

4.6 Soil enzyme activities

Dehydrogenase activity (DHA) in rice soils ranged from 2.5 to 11.2 µg (TPF) g^{-1} (dw) h^{-1} and the highest activity was observed in soils of Saiden lowland followed by Kyrdemkulai lowland and the lowest was recorded in soils of Saiden upland terraces (Fig. 4.2). DHA was significantly higher in post-winter in all rice fields except the lowland rice fields where seasonal variation of DHA was not non-significant.

Phosphatase activity (PHA) in rice soils ranged from 4.3 to 12.0 µg (PNP) g^{-1} (dw) h^{-1} and the highest activity was observed in soils of lowland rice fields and the lowest was recorded in soils of Saiden upland terrace-1 (Fig. 4.3). PHA was significantly higher in post-winter in all rice fields as compared to that in post-summer.

4.7 Comparison of rice ecosystems in terms of soil C, N and P fractions

The six rice fields those represented three distinct rice ecosystems were plotted in two dimensions by performing PCA considering all fractions of C, N, and P including pH, DHA and PHA either in post-summer or in post-winter are presented in the Fig. 4.4 and 4.5, respectively. In post-summer time, PCA plot generated three distinct clusters at Euclidean distance 5.7 (Fig. 4.4). The cluster consisted of Kyrdemkulai lowland and upland terrace rice fields was distinctly separated from the cluster consisted of Saiden upland rice fields by the PC1 that explained variability of 48.7%. The third cluster only consisted of Saiden lowland was distinctly separated by the PC1 and PC2 from the cluster of Saiden upland rice fields, whereas it was separated only in one direction by the PC2 (variability of 16.2%) from the cluster of Kyrdemkulai lowland and upland terrace rice fields.

In post-winter time, four distinct clusters were formed in the PCA plot at Euclidean distance 4.9 (Fig. 4.5). The lowland rice fields (Saiden lowland and Kyrdemkulai lowland) and Saiden slope land, each formed individual cluster yielding three distinct clusters in the PCA plot. Whereas, Kyrdemkulai upland

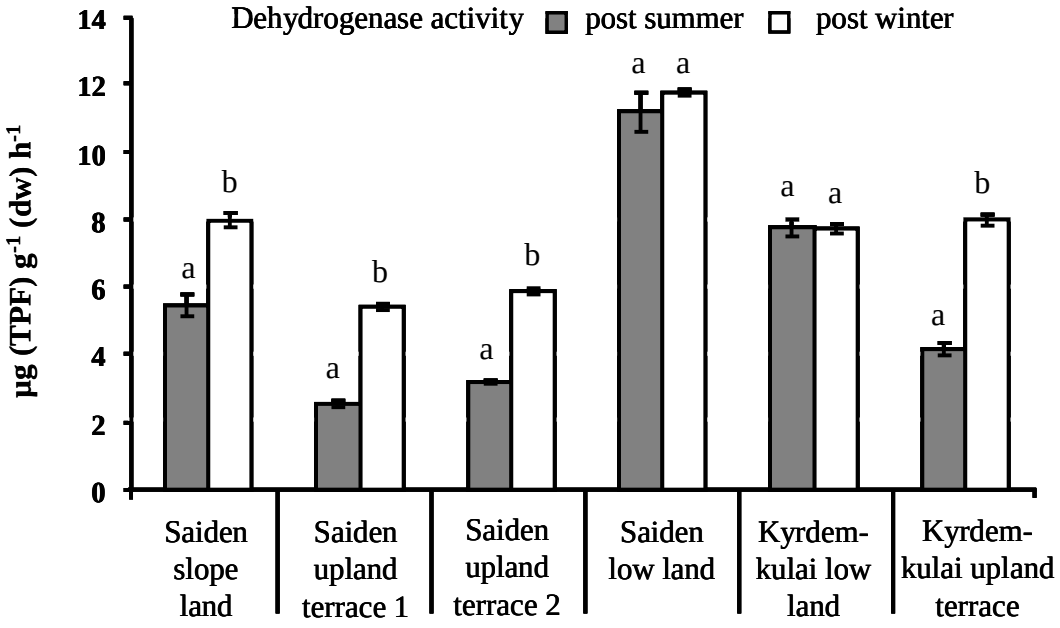

Fig. 4.2 Dehydrogenase activity in soils of different rice ecosystems as influenced by seasons. Within a site, mean values (columns) with different letters were significantly different (*P*<0.05).

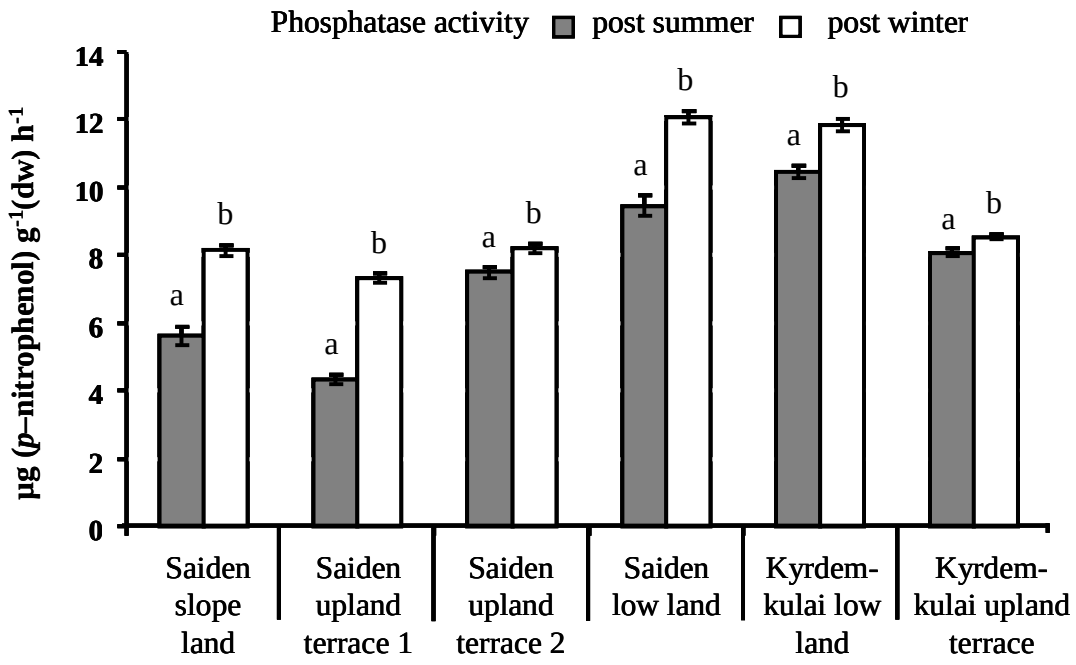

Fig. 4.3 Phosphatase activity in soils of different rice ecosystems as influenced by seasons. Within a site, mean values (columns) with different letters were significantly different (*P*<0.05).

terrace and Saiden upland terrace 1 and 2, all together formed the forth cluster. All these four clusters were separated from each other by PC1 and PC2. However, the distance between the cluster of Saiden slope land and the cluster consisted of Kyrdemkulai and Saiden upland terraces were much closer in comparison to that from the clusters of Saiden and Kyrdemkulai lowlands. Differences among rice fields expressed in the PCA plots were due to the underlying variations existed among the fractions of C, N, and P, which was evident from the correlation coefficients of the linear combinations of all the parameters under consideration with PC1 and PC2 (See appendix 1.1 and 1.2).

4.8 Relationship among various fractions of soil C, N and P

Season-wise all fractions of C, N, and P were analysed for pair-wise correlations among themselves, irrespective of rice fields and the data are presented in the Table 4.6 and 4.7 for post-summer and post-winter, respectively. TOC, TN and TP exhibited significant positive correlation with each other in both seasons. DOC maintained a strong positive correlation with TOC (r = 0.92 in post-summer and r = 0.95 in post-winter; $P<0.001$) and also significant positive correlation with TN and TP. A significant positive correlation was observed between DOC and pMN in both seasons. However, DOC did not show strong positive correlation with Avl-N in post-summer, but in post-winter correlation was strong (r = 0.64, $P<0.01$). DOC showed strong positive correlation (r = 0.90, $P<0.001$) with EON in post-winter, whereas the relationship was very week (r = 0.22, $P>0.05$) in post-summer season. MBC was found to be significantly correlated with TOC, DOC, TN, pMN, TP, MBN, EON, MBP, DHA and PHA ($P<0.05$ and <0.01), irrespective of seasons. EON maintained strong positive correlation (r = 0.39 to 0.95, $P<0.05$ to 0.01) with TOC, DOC, MBC, TN, MBN and MBP in both seasons, except the correlations with DOC and Avl-N (r = 0.22 and 0.32, respectively $P>0.05$) in post-summer. MBN and MBP showed strong positive correlation (r = 97 and o.99 for post-summer and post-winter,

respectively) in rice soils. Soil pH maintained strong negative correlation with all fractions of C, N, and P, except Avl-P, TP and pMN in post-summer. DHA and PHA maintained a significant negative correlation with soil pH, irrespective of seasons. However, soil pH did not show strong negative or positive correlations with the fractions of C, N, and P in post-winter, except Avl-N, pMN and DHA. Avl-N and DHA negatively correlated with soil pH, whereas pMN positively correlated with soil pH. Avl-P maintained significant positive correlation with TP in post-winter, but same relationship was not significant in post-summer. Similarly, no significant positive correlation was observed between Avl-N and TN in rice soils. MBN, MBP, DHA and PHA maintained significant positive correlation among each other ($r = 0.43$ to 0.91, $P<0.01$).

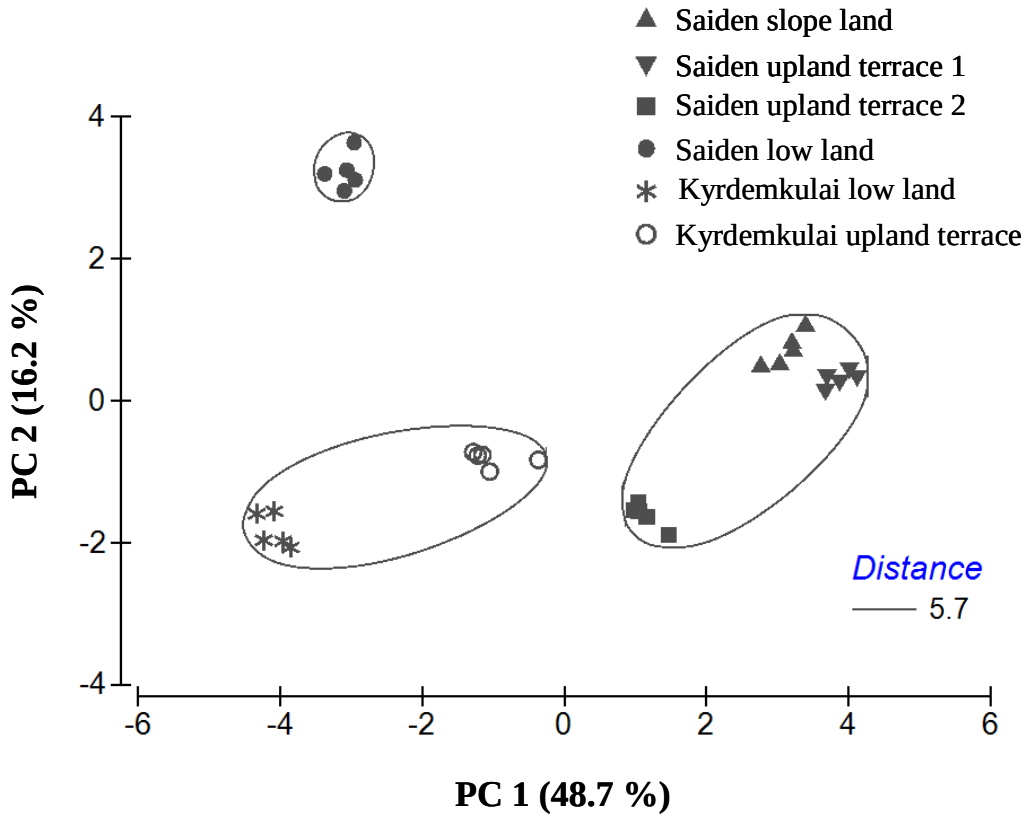

Fig 4.4 Comparison of variability (in terms of pH and various fractions of C, N and P, and soil enzyme activities) among different rice ecosystems in post-summer performed by principal component analysis. Data were square root transformed and normalized to eliminate the effects of units. Principal axes 1 and 2 expressed variability of 48.7% and 16.2%, respectively. Ellipses represent superimposed hierarchical clusters (Euclidean distance 5.7) based on Bray-Curtis similarity matrix, deduced using group-average linking incorporating similarity of profile (SIMPROF) at 95% confidence limit.

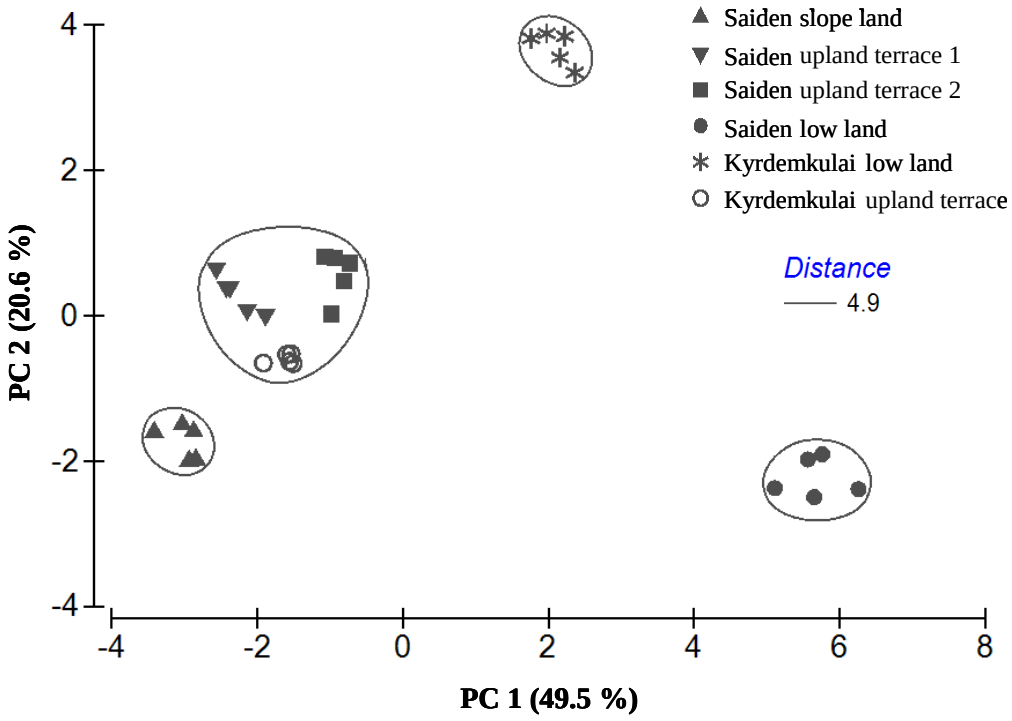

Fig 4.5 Comparison of variability (in terms of pH and various fractions of C, N and P, and soil enzyme activities) among different rice ecosystems in post-winter performed by principal component analysis. Data were square root transformed and normalized to eliminate the effects of units. Principal axes 1 and 2 expressed variability of 49.5% and 20.6%, respectively. Ellipses represent superimposed hierarchical clusters (euclidean distance 5.7) based on Bray-Curtis similarity matrix, deduced using group-average linking incorporating similarity of profile (SIMPROF) at 95% confidence limit.

Table 4.6 Pair-wise relationship between parameters of carbon, nitrogen and phosphorus in rice ecosystems during post-summer season

Parameter	pH	TOC	DOC	MBC	BAS	TN	Avl-N	MBN	EON	pMN	TP	Avl-P	MBP	DHA	PHA
pH	1														
TOC	-0.74	1													
DOC	-0.51	0.92	1												
MBC	-0.75	0.97	0.92	1											
BAS	-0.32	0.57	0.49	0.57	1										
TN	-0.47	0.80	0.82	0.72	0.69	1									
Avl-N	-0.48	0.48	0.17	0.30	0.34	0.34	1								
MBN	-0.82	0.94	0.83	0.96	0.70	0.76	0.40	1							
EON	-0.89	0.44	0.22	0.45	0.27	0.39	0.32	0.61	1						
pMN	-0.21	0.51	0.54	0.57	0.94	0.64	0.02	0.66	0.16	1					
TP	0.07	0.45	0.45	0.42	0.79	0.45	0.35	0.43	-0.31	0.74	1				
Avl-P	-0.04	-0.24	-0.51	-0.23	0.38	-0.29	0.39	-0.03	0.14	0.22	0.29	1			
MBP	-0.82	0.95	0.85	0.99	0.54	0.65	0.35	0.97	0.53	0.52	0.36	-0.14	1		
DHA	-0.57	0.75	0.52	0.68	0.78	0.57	0.78	0.77	0.32	0.58	0.73	0.41	0.71	1	
PHA	-0.65	0.94	0.90	0.88	0.72	0.95	0.43	0.91	0.47	0.66	0.49	-0.22	0.84	0.72	1

[TOC - total organic carbon, DOC - dissolved organic carbon, MBC - microbial biomass carbon, BAS - basal respiration, TN – total nitrogen, Avl-N – available nitrogen, MBN – microbial biomasss nitrogen, EN – extractable organic nitrogen, pMN – potentially mineralizable nitrogen, TP – total phosphorous, Avl-P – available phosphorous, MBP – microbial biomasss phosphorous, DHA – dehydrogenase activity, PHA – phosphatase activity.]

* significant at $r_{0.05}$= 0.36 and **significant at $r_{0.01}$= 0.46

Table 4.7 Pair-wise relationship between parameters of carbon, nitrogen and phosphorus in rice ecosystems during post-winter season

Parameter	pH	TOC	DOC	MBC	BAS	TN	Avl-N	MBN	EON	pMN	TP	Avl-P	MBP	DHA	PHA
pH	1														
TOC	-0.15	1													
DOC	-0.18	0.95	1												
MBC	0.15	0.86	0.86	1											
BAS	0.32	-0.19	-0.22	0.25	1										
TN	-0.10	0.83	0.66	0.65	-0.07	1									
Avl-N	-0.69	0.54	0.64	0.39	-0.32	0.11	1								
MBN	-0.03	0.89	0.93	0.96	0.05	0.55	0.62	1							
EON	-0.16	0.92	0.90	0.86	-0.14	0.59	0.71	0.95	1						
pMN	0.43	0.50	0.51	0.61	0.21	0.63	-0.28	0.44	0.24	1					
TP	0.00	0.60	0.73	0.66	0.07	0.52	0.19	0.62	0.41	0.84	1				
Avl-P	-0.12	0.03	0.22	0.00	-0.12	0.08	-0.07	-0.01	-0.21	0.57	0.75	1			
MBP	-0.02	0.85	0.91	0.94	0.07	0.47	0.64	0.99	0.93	0.40	0.60	-0.01	1		
DHA	-0.53	0.57	0.70	0.38	-0.29	0.49	0.48	0.45	0.37	0.48	0.82	0.75	0.43	1	
PHA	-0.33	0.95	0.98	0.83	-0.19	0.72	0.68	0.90	0.88	0.47	0.72	0.22	0.86	0.75	1

[TOC - total organic carbon, DOC - dissolved organic carbon, MBC - microbial biomass carbon, BAS - basal respiration, TN – total nitrogen, Avl-N – available nitrogen, MBN – microbial biomasss nitrogen, EN – extractable organic nitrogen, pMN – potentially mineralizable nitrogen, TP – total phosphorous, Avl-P – available phosphorous, MBP – microbial biomasss phosphorous, DHA – dehydrogenase activity, PHA – phosphatase activity.]

* significant at $r_{0.05}$= 0.36 and **significant at $r_{0.01}$= 0.46

CHAPTER 5

Discussion

In the recent years, it has been realized that understanding the biological pools of C, N, and P in soils of agro-ecosystems and their seasonal dynamics are crucial in managing or altering nutrient availability in soils and judging the nutrient supplying capacity of the soils. Seasonal dynamics and sizes of biological pools of C, N, and P in soils of different hill rice ecosystems of NE India are not characterized earlier. So, the present study assessed the biological pools of C, N, and P in soils of three rice ecosystems viz. direct-seeded hill slope, transplanted upland terrace and transplanted lowland. Findings revealed that soils of different rice ecosystems markedly varied in several biological parameters of C, N, and P especially in terms of pool sizes and seasonal dynamics.

Difference between rice ecosystems were pronounced in terms of soil moisture status. The lowland rice fields were waterlogged and maintained an anaerobic situation throughout the year, and supported a diverse aquatic weed community and produced large quantity of above- and below-ground biomass. Soils of lowland rice fields contained a dense root mat up to few centimeters below from the soil water interface. Soil moisture content in slope land rice field (Saiden slope land) was low (13.9-28.1%) in both post-summer and post-winter due to lack of moisture conservation strategies. This ecosystem is aerobic in nature. Similarly, soil moisture content in Saiden upland terrace-1 was also low (32%) in both seasons. This terrace land was newly constructed (1-year old) and not yet recovered from its physical, chemical and biological alterations. Saiden upland terrace-2 contained soil moisture at optimum levels i.e. 60% in post-summer and 53.2% during post-winter. This terrace land was constructed 20 years back and the soil supported a large quantity of above- and below-ground weed biomass. Soil moisture content in Kyrdemkulai upland terrace differed significantly between seasons (61.4% in post-summer and 22.0% in post-winter).

This terrace was constructed 7 years back and supported relatively a less diverse above- and below-ground weed biomass compared to that in lowland rice ecosystems. Results revealed that all the rice fields varied distinctly in terms of seasonal dynamics of soil moisture and oxygen status, and above- and below-ground biomass.

Seasonal fluctuation in soil pH was observed in lowland and upland terrace land, whereas soil pH in slope land was stable in both seasons. The cause of low soil pH in post-summer in comparison to that in post-winter in lowlands and upland terraces might be the presence of organic acids in soil solution during anaerobic decomposition of weed biomass under high temperature and excessive moisture conditions in post-summer. Such activities considerably reduced with the decrease in soil temperature in post-winter and hence, increase in soil pH was noticed. This was evident because of low BAS in soils of all rice ecosystems in post-winter as compared to that in post-winter (Table 4.2). Brady and Weil (2007) reported that the fluctuation in soil pH between summer and winter is because of the movement of salts into and out of the soil zones as the soil moisture moves up and down through the soil profile and the other reason is the intense organic matter decay with onset of warm temperature and rain. But change in soil pH was not significant in Saiden upland terrace-2, which might be due to low soil moisture content in both seasons. In the case of slope land, soil pH was low compared to other rice ecosystems, which might be due to soil erosion and removal of base forming cations due to excessive runoff of rain water.

Finding revealed that rice ecosystems are markedly different in terms of TOC, DOC and MBC contents in soils. The large quantities of above- and below-ground biomass (including both aquatic weeds and rice roots) produced during summer contributed towards TOC pool in soils of rice ecosystems; however amount of such contribution is relatively higher in lowland rice soils leading to higher TOC as compared to that in upland terrace and slope land rice soils. The lower content of TOC in slope land soils in post-winter in comparison to that in post-summer might be the impact of burning of above-ground biomass in slope land coupled with the loss of surface soil layer due to erosion. The comparative

lower amount of TOC in post-summer that in post-winter in rice soils (exception was the Saiden slope land) might be due to gradual accumulation of large amount of partially decomposed organic matter during anaerobic decomposition in summer season (Brady and Weil, 2007).

Diverse substrate availability and moisture content in soils of lowlands round the year supported higher MBC in comparison to that in other rice ecosystems. Lu *et al.* (2002) also reported that the MBC dynamics in rice soils largely controlled by the organic substances released by root community. It was reported that microbial community of lowland rice soils predominantly composes of facultative and anaerobic populations which is markedly different from aerobic populations in upland rice soils (Reichardt *et al*., 2001). The low soil moisture content was responsible for decrease of MBC in Saiden slope land and Kyrdemkulai upland terrace in post-winter. MBC content in soils maintains significant positive correlations with soil moisture content and mean air temperature (Devi and Yadava, 2006).

DOC pool is a leaching product from plants, litter and humus and it is generated by microbial activity (McDowell and Likens, 1988; Kalbitz *et al*., 2000); this might be the reason for higher DOC in lowland soil as it supports diverse above- and below-ground biomass and higher TOC. Irrespective of seasons, a significant positive correlation ($r = 0.92$ in post-summer and 0.86 in post-winter; $P<0.01$) between MBC and DOC indicated the direct role of MBC in controlling DOC in soils. Similarly, DOC content in soils also maintained a significant positive correlation with soil moisture content ($r = 0.85$, $n=30$, $P<0.01$). Similar observation was also reported by Christ and David (1996).

BAS is the steady rate of respiration in soil originated from the turnover of organic matter and the rate reflects the potential of soil biota component to decompose soil organic residues (Pell *et al*., 2006). BAS and MBC showed a significant positive correlation in post-summer, whereas the relationship was weak in post-winter. The reason for this might be due to low soil temperature in post-winter. Jia and Zhou (2009) reported that within an optimum soil moisture range, BAS is positively correlated with soil temperature. The high soil

respiration especially during summer is attributed to higher rainfall and temperature which supported higher microbial biomass leading to enhanced soil microbial activities as reported by Laik *et al*. (2009). This finding was further validated by comparative analysis of glucose induced soil respiration in soils of different rice fields during initial 7 days of incubation. Results clearly reflected that glucose induced respiration was significantly lower in post-winter compared to that in post-summer, irrespective of rice fields. Though glucose induced respiration was determined at constant temperature of 22^0C at laboratory, it might be possible that inactive members of the soil microbial community due to low temperature in post-winter need a lag period to be activated. It was reported that on substrate availability soil respiration increases due to activation of certain groups of microbes resulting in microbial CO_2 production which is proportional to the mass of organisms (Hoper, 2006; Bailey *et al*., 2008).

Approximate 2 times less TN content in soils of Saiden slope land and Saiden upland terrace-1 compared to that in lowland rice fields indicated that disturbances to ecosystem had significant negative impact on TN content. Saiden slope land was under the influence of periodic burning and exposed to soil erosion and on the other hand Saiden upland terrace-1 was newly constructed (1-year old). TN content in soils is highly dependent on the degree of soil disturbance or tillage systems (Ren and Hu, 2010). Higher TN content in soils irrespective of rice field types in post-winter might be the result of gradual accumulation of summer grown above- and below-ground biomass in soils. Revelation of the results on EON, Avl-N, MBN and pMN indicated that stability of the ecosystem and moisture content in soils had significant influences on sizes of labile-N pools in rice ecosystems. Less disturbed rice ecosystems like Saiden and Kyrdemkulai lowlands and Saiden upland terrace-2 (20 years old) supported larger amount of labile-N fractions (EON, Avl-N and MBN) and higher rate of pMN compared to that in other disturbed rice ecosystems like Saiden upland terrace (1-year old) and slope land rice fields. Similarly, lower amount of EON, Avl-N and MBN also coincided with low soil moisture content in soils of Saiden slope land and Kyrdemkulai upland terrace in post-winter (Table 4.3). Another important finding

was that sizes of labile-N pools were significantly higher in post-winter compared to that in post-summer in all rice fields except the disturbed rice fields {Saiden upland terrace (1-year old) and slope land}. Reasons behind are: 1) dilution effects on labile-N fractions due to excessive soil moisture and 2) uptake of large proportion of available fraction of N by the summer grown biomass in rice field. It was previously reported that differences in EON content between seasons are due to differences in temperature and moisture which controls the sizes and activities of microbiota community and its adaptation (Schmidt *et al*., 2007). Again, labile-N fractions are highly dependent on the inputs from the rhizosphere, change in land use and biomass content (Appel and Mengel, 1998; Haynes, 2005; Ros *et al*., 2009). The higher amount of MBN, EON and Avl-N in slope land in post-summer corroborate the finding that increase in Avl-N in slope land during summer is because of biomass burning in preceded winter season under jhuming practice (Venkatesh *et al*., 2001).

MBN is higher in post-winter than in post-summer, because MBN tended to decrease during the rainy season whereas they tended to increase and remain at high levels during the dry season as also found by Sugihara *et al*. (2010) and increased in soil fauna during the rainy season increases grazing on soil microbes, thereby reducing microbial biomass (Halverson *et al*., 2000; Michelsen *et al*., 2004). So, we can say that soil moisture strongly affected the seasonal variations in labile-N fractions and soil microbes acted as a nutrient source during the rainy season and as a sink in the dry season.

pMN indicated the rate of mineralization of active fraction of organic N through microbial action in soils and that it gives an idea on N supplying capacity of the soil for crop growth (Doran and Parkin, 1994). Results of pMN indicated that N supplying capacity of lowland rice soils was higher than the upland terrace and slope land rice fields. Soil moisture below optimum level, for example 22% in Kyrdemkulai upland terrace in post-winter reduced pMN in rice soils; otherwise, pMN in post-winter season was found higher in all other rice soils. The reduction in saturation of wetland soils during winter has frequently been associated with increases in both net mineralization and nitrification. Reduction in

moisture below saturation allow aerobic conditions to become established as many observations of drained wetlands have found increases in both soluble inorganic N and N process rates (Olde Venterink *et al*., 2002; Tiemeyer *et al*., 2007) or the maximum rate mineralization rate occurs near field water holding capacity.

TP content in rice soils did not fluctuate significantly between seasons, because the turnover of the P content in soil is very slow to be identified within the short period of time. TP content in soil of slope land was approximate 2 times less than that in other rice fields, which might be due to burning of biomass during winter and loss of surface layer due to erosion during summer. However, Avl-P pool size was higher in Saiden slope land in post-summer because of higher microbial activity coupled with increase in readily available P fraction due to biomass burning in preceded winter. Venkatesh *et al*. (2001) previously reported that biomass burning during winter increases Avl-P fractions in soils under jhuming practices.

In most lowland rice soils, P availability initially increases upon flooding (Ponnamperuma, 1972; Willett, 1986). In this investigation, it was observed that Avl-P fraction in soils of lowland rice was less in summer compared to that in winter. The inorganic fraction of P might get immobilized due to uptake by the actively growing aquatic biomass during summer. Again, high concentrations of reduced forms of Fe and Al might complex with Avl-P fraction. This might be the cause for poor correlation observed between MBP and Avl-p in rice soils. MBP and MBN showed significant positive correlation indicating higher MBP in post-winter than that in post-summer and among the rice fields higher MBP in lowland.

DHA is an important indicator parameter to study biological activity of agricultural soils including flooded soils (Chendrayan *et al*., 1980; Nannipieri *et al*., 2002; Wlodarczyk *et al*., 2002). Higher MBC content in soils of lowland among rice fields and in post-winter between seasons might lead to higher DHA activity under these situations. Decreased redox potential in lowlands showed higher DHA activity (Makol *et al*., 2008). Higher phosphatase activity in soils

coincided with higher contents of TP, Avl-P and microbial biomass in soils (Chhonkar and Tarafdar, 1984). Similar relationships were also observed in rice ecosystems except PHA did not correlate with Avl-P in rice soils. This might be due to complex dynamics of Avl-P in acidic soils with high Fe and Al activities under different moisture levels.

MBC, MBN and MBP contributions to TOC, TN and TP, respectively were high in lowland rice soils compared to other rice ecosystems. This finding clearly showed that MBC, MBN and MBP act as a major sink in replenishing pools of C, N and P in lowland rice ecosystems. Soil microbial biomass has an average C:N:P ratio of ~ 50:6:1 (Smith and Paul, 1990). Findings revealed that C:N:P ratio in rice soils varied from as low as 35.3:4.9:1 in lowland rice soils to a maximum of 54:6.5:1 in Kyrdemkulai upland terrace in post-winter. So, the mineralization potential of C, N and P is higher in lowland rice soils as compared to that in upland rice soils.

The cycling of C, N and P occur concurrently in soil. So, comparative assessment of soils of different agro-ecosystems at a single point of time considering various fractions of C, N and P together is much more meaningful. Principal component analysis was carried out considering all biological pools of C, N and P analysed in this study as variables to examine the grouping behaviour of different rice fields depending on seasons. In the PCA plot, Kyrdemkulai lowland and upland terrace grouped together in post-summer due to little or no variations in biological pools of C, N and P. Similarly, Saiden lowland though grouped alone, but it was more closed to Kyrdemkulai lowland group in comparison to the group formed by Saiden upland terrace and slope land in post-summer. Interestingly, PCA plot generated for post-winter indicated that Kyrdemkulai upland terrace grouped with the Saiden upland terraces. The change in grouping behaviour of Kyrdemkulai upland terrace was due to large seasonal variability in biological pools of C, N and P according to soil moisture status. These findings clearly indicated that biological pools of C, N and P of rice fields behaved variably depending on soil moisture status and that override the impact of location on biological pools of C, N and P.

Results of the pairwise correlations among the fractions of C, N and P analysed in this investigation were interrelated, irrespective of types of rice fields with only exceptions between Avl-N with pMN, EON with pMN, Avl-N with TN, BAS with Avl-N or EON, Avl-P with MBP, Avl-P with PHA. MBC is the living and the most active part of soil organic carbon (SOC) and give information on the formation and turnover of SOC by soil microbiota (Powlson *et al*., 1987; Friedel *et al*., 1996) and it shows strong positive correlation with SOC both in winter and summer seasons (Ingram *et al*., 2005). Several researchers earlier showed that there is a significant linear relationship between MBC and SOC in temperate environments and this relationships exist in tropical environments as well where the SOC levels are very low (Jenkinson and Ladd, 1981; Leita *et al*., 1999; Banerjee *et al*., 2006). DOC is considered as the most active and mobile phase which play major role in the transport and supply of C and N to microbial populations (Jones *et al*., 2004; Cookson *et al*., 2005). The production and composition of DOM is largely dependent on its equilibrium with total SOM (Gregorich *et al*., 2000). In this study DOC showed direct correlation with MBC or TOC, which was also reported by Cookson *et al*. (2008) and suggested that there is considerable cycling of C between these pools. DOC correlated significantly with TOC, MBC, TN, MBN, pMN, TP, MBP, DHA and PHA in both post-summer and post-winter. However, DOC showed a significant negative correlation with Avl-P in post-summer and this relationship changed to positive direction through not significant in post-winter. DOC maintained a significant positive correlation with soil moisture content ($r = 0.86$, $n= 30$, $P<0.01$) both in post-summer and post-winter indicating an increase in DOC production with increase in soil moisture, which was also observed earlier (Christ and David, 1996). Lu *et al*. (2004) reported that DOC plays important roles in nutrient cycling and methane production in flooded rice ecosystem. BAS has direct relationship with TOC. Zheng *et al*. (2009) for soil respiration as active decomposition of soil carbon matter and the enhancement of plant-derived CO_2 release from root respiration, and results in a quick increase in the soil CO_2 efflux

rate. It also shows positive correlation with moisture during summer and does not correlate during winter season which shows that with optimum moisture it also related to temperature as reported by Laik *et al*. (2009).

In general, amount of N mineralized in soils is correlated with TN, TC, MBN, and clay content (Ireneo *et al*., 1996; Li *et al*., 2003). Rasmussen *et al*. (1998) also reported that pMN significantly correlated with TN. Findings of this study further validated similar observations in rice ecosystems. pMN showed weak negative correlation with soil pH in summer but maintained a direct relationship in winter. As soil pH decreases the microbial activity and decomposition is also affected (Paul *et al*., 2001). But N mineralization is often reported to be relatively insensitive to changes in pH, occurring over a wide range (Paul and Clark, 1996). MBN showed strong positive correlation with TOC in this study, which corroborate with the previous findings that the increase in soil organic carbon content, MBC and N increased in the long-term experiment (Zhong and Cai, 2007). Avl-N maintained a direct relationship with MBN, EON and DOC in rice soils in both seasons. Recently, Steenwerth and Belina (in press) reported direct relationship between inorganic N fraction with dissolved organic N and C. In this study, above- and below-ground biomass in rice soils might contribute to DOC and EON pools. The direct relationship between Avl-N and MBN indicated that a significant proportion of Avl-N portion comes from the N-mineralization process in water body in rice field instead of soil layer and hence, Avl-N and MBN didn't show opposite relationship. So, NH_4-N supply from water body as a byproduct of biological-N fixation in rice is an important pool. Size of EON pool is dependent on TN as EON pool significantly correlated with TN. Similar observation was previously reported by by Ros *et al*. (2009).

DHA and PHA maintained strong positive correlation in rice soils (Table 4.6 and 4.7) and similar relationship was previously reported by many authors (Garcia *et al*., 1996; Kandeler *et al*., 1999; Dilly and Nannipieri, 2001).

CHAPTER 6

Summary

Soil is a complex dynamic medium and is a reservoir of essential plant nutrients. Availability of these nutrients in soil is regulated by the activities of soil biota communities. The land use type and agricultural management practices have significant impacts on community composition and functioning of soil biota communities. Again, soil C plays a key role in maintenance of soil biota community. Soil C is known to interact with N and P during the residue decomposition and changes in land use practices can have a marked effect on these contents. So, assessment of biological pools of C, N and P are important in understanding the functioning of an ecosystem for its sustainability.

Improper soil conservation measures coupled with high rainfall in North Eastern India cause depletion of soil organic matter leading to heavy soil degradation. Three distinct rice ecosystems namely slope land, upland terrace and lowlands are common in hill states of NE India and these rice ecosystems are different in terms of soil type, topography, water content, above- and below-ground biomass and anthropogenic activities. Keeping above views in mind, the present investigation was formulated to characterize biological pools of C, N and P in soils of three distinct rice ecosystems located in Saiden and Kyrdemkulai villages of Ri-Bhoi district in Meghalaya.

Findings of this investigation are summarized below:

- Differences in soil moisture between rice ecosystems were distinct. Lowland rice soils were waterlogged and predominantly anaerobic in both post-summer and post-winter. Slope land and young upland terrace rice soils were deficient in soil moisture in both sampling times. Seasonal fluctuation in soil moisture was pronounced in upland terrace rice fields indicating deficient soil moisture in post-winter.

- Soil pH in rice ecosystems observed in the range of 4.8 to 5.6. The observed pH was in the order of Slope land < lowland < upland terrace.
- Finding revealed that rice ecosystems are markedly different in terms of TOC, DOC and MBC contents in soils. TOC, MBC and DOC in soils were observed in the order of slope land and young upland terrace < mature upland terrace < lowland. TOC, DOC and MBC contents in rice soils maintained maintained significant positive correlation with each other. Seasonal fluctuation of TOC, MBC and DOC was pronounced indicating significant higher values in post-winter in all rice field with only exception was in slope land.
- BAS was significantly higher in post-summer compared to that in post-winter in all rice fields indicating higher biota activities in soils in summer. Similarly, glucose induced soil respiration indicated higher biota activitiy in summer compared to that in winter. Lowland rice showed higher levels of BAS compared to other rice fields.
- TN in soils of rice ecosystems observed in the order of slope land and young upland terrace < mature upland terrace < lowland. Increase and decrease of total N depends on the soil disturbance or tillage system.
- Revelation of the results on EON, Avl-N, MBN and pMN indicated that stability of the ecosystem and moisture content in soils had significant influences on sizes of labile-N pools in rice ecosystems. Less disturbed rice ecosystems like Saiden and Kyrdemkulai lowlands and Saiden upland terrace-2 (20 years old) supported larger amount of labile-N fractions (EON, Avl-N and MBN) and higher rate of pMN compared to that in other disturbed rice ecosystems like Saiden upland terrace (1-year old) and slope land rice fields. Low soil moisture content in post-winter severely affected the EON and MBN.
- TP in soils of rice ecosystems observed in the order of slope land and young upland terrace < mature upland terrace < lowland. Avl-P pool showed poor correlation with TP or MBP in all rice fields and this might be due complex chemistry of Fe and Al present abundantly in rice soils. Over all, Avl-P status in rice soils was found to be the major limiting factor in rice soils.

- MBP and MBN showed significant positive correlation indicating higher MBP in post-winter than that in post-summer and among the rice fields higher MBP in lowland.
- Lowland rice soils exhibited higher DHA and PHA activities compared to that in other rice fields.
- Findings revealed that C:N:P ratio in rice soils varied from as low as 35.3:4.9:1 in lowland rice soils to a maximum of 54:6.5:1 in upland terrace rice soils. So, the mineralization potential of C, N and P is higher in lowland rice soils as compared to that in upland rice soils.
- Principal component analysis performed considering all analysed biological pools of C, N and P as defined variables indicated that each rice field grouped distinctly. The similarity between rice fields indicated three distinct clusters in post-summer and four distinct clusters in post-winter. These clusters were according to lowland, upland terrace and slope land rice ecosystems indicating that biologiocal pools C, N and P of three rice ecosystems very differed significantly. However, the change of grouping behavior of Kyrdemkulai upland terrace (grouping with Kyrdemkulai lowland in post-summer and grouping with Saiden slope land and upland in post-winter) in the PCA plot revealed that large seasonal variability in biological pools of C, N and P according to soil moisture status was major factor. So, biological pools of C, N and P of rice fields behaved variably depending on soil moisture status and that overrided the impacts of location on biological pools of C, N and P.
- Pair-wise correlation matrix analysis indicated that various biological pools of C, N and P maintained close relationship, irrespective of rice field types.
    - TOC, DOC and MBC showed significant positive correlation between each pair.
    - DOC correlated significantly with TN, MBN, pMN, TP, MBP, DHA and PHA.
    - DOC showed negative correlation with Avl-P and this relationship was significant in post-summer.

- DOC maintained a significant positive correlation with soil moisture content ($r = 0.86$, $n= 30$, $P<0.01$) both in post-summer and post-winter.
- BAS showed direct relationship with TOC in soil.
- pMN in soils showed strong positive correlated with TN, TOC, MBC, MBN and MBP. However, pMN showed weak negative correlation with soil pH in summer but maintained a direct relationship in winter. Avl-N maintained a direct relationship with MBN, EON and DOC in rice soils in both seasons.
- Direct relationship between Avl-N and MBN in rice soils indicated that a significant proportion of soil Avl-N water body present in rice soil through N-mineralization process instead of soil layer. So, NH_4-N supply from water body as a byproduct of biological-N fixation in rice ecosystem is an important N-pool. Size of EON pool is dependent on TN as EON pool significantly correlated with TN.
- DHA and PHA maintained strong positive correlation between each other in rice soils. Each of DHA and PHA strongly correlated with TOC, DOC, TN, TP, EON, MBC MBN, MBP and string negative correlation with soil pH.

CHAPTER 7

Conclusion

The three common rice ecosystems viz. slope land, upland terrace and lowland prevailed in hilly states of North East India varied greatly in terms of biological pools of C, N and P in soils, and these pools were strongly influenced according to soil moisture status. The DOC plays a very important role in mineralization process and the rate of mineralization. The stable lowland ecosystem had higher potentially mineralizable N than disturbed ecosystem. This indirectly reflects the biological cycle disturbance in disturbed ecosystem. Even though there might be lesser mineralization in waterlogged soils but immobilization is also lower, so net mineralization is high. The C:N:P ratio in rice soils varied from as low as 35.3:4.9:1 in lowland rice soils to a maximum of 54:6.5:1 in upland terrace rice soils indicating high mineralization potential of C, N and P in lowland rice ecosystem than upland and slope land rice ecosystems. Various biological pools of C, N, and P viz. MBC, DOC, EON, pMN, MBN, MBP were strongly correlated with each other. Sizes of these pools were strongly dependent on the total pools of C, N and P in rice soils. DOC and EON were also highly correlated with soil moisture status. Avl-P showed poor correlation with TP or MBP in all rice fields and Avl-P was found to be the major limiting factor in rice soils. Biological pools of C, N and P of rice fields behaved variably according to ecosystem type and soil moisture status and such effects overrided the impacts of locational difference on biological pools of C, N and P. Therefore, management of moisture in soils of rice ecosystems is an important aspect for maintaining balanced biological pools of C, N and P. Again, emphasis should be given in developing site-specific nutrient management package in rice fields according to rice ecosystem types and soil moisture status. Overall, it can be concluded that C and N components of rice soils seem to be self-sustained in lowland and stabilized upland terrace rice ecosystems, but the major limiting factor was availability of P. For the first time, this study has standardized the methods of analysis of biological pools of C, N and P in hill soils of NE region of India.

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Appendix

Appendix 1.1 Principal component analysis performed using various fractions of C, N and P as defined variables in post-summer

A. Eigen values

Principal component	Eigen values	% variation	Cumulative % variation
PC1	9.25	48.7	48.7
PC2	3.09	16.2	64.9
PC2	2.9	15.3	80.2

B. Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variables	PC 1	PC2	PC3
pH_S	0.181	-0.148	0.399
MBC_S	-0.312	0.061	-0.032
TOC_S	-0.291	0.016	-0.190
DOC_S	-0.289	0.139	0.192
BAS_S	-0.226	-0.272	-0.022
SIR_1S	0.006	-0.429	0.174
SIR_2S	-0.259	-0.027	0.169
SIR_3S	-0.253	0.215	0.129
MBN_S	0.129	0.084	-0.128
Avl N_S	-0.120	-0.216	-0.310
TN_S	-0.300	0.081	-0.147
EON_S	-0.121	0.169	-0.467
pMN_S	-0.233	-0.219	0.099
TP_S	-0.186	-0.408	0.208
AP_S	0.058	-0.462	-0.290
MBP_S	-0.201	0.059	0.404
DHA_S	-0.245	-0.299	-0.173
PHA_S	-0.314	0.050	-0.031

[TOC - total organic carbon, DOC - dissolved organic carbon, MBC - microbial biomass carbon, BAS - basal respiration, SIR – Substrate induced respiration, TN – total nitrogen, Avl-N – available nitrogen, MBN – microbial biomasss nitrogen, EN – extractable organic nitrogen, pMN – potentially mineralizable nitrogen, TP – total phosphorous, Avl-P – available phosphorous, MBP – microbial biomasss phosphorous, DHA – dehydrogenase activity, PHA – phosphatase activity. S denotes post-summer]

C. PCA scores

Sites	Score 1	Score 2	Score 3	Name of sites
Site1_1	3.040683	-0.51263	-3.33208	Saiden slope land
Site1_2	2.775101	-0.48315	-3.33703	
Site1_3	3.212248	-0.81188	-3.16755	
Site1_4	3.404231	-1.05778	-2.81322	
Site1_5	3.231433	-0.69856	-3.01114	
Site2_1	4.129062	-0.35382	2.312787	Saiden upland terrace 1
Site2_2	4.020908	-0.45866	2.1593	
Site2_3	3.883188	-0.28554	1.909096	
Site2_4	3.682515	-0.15591	1.678989	
Site2_5	3.704544	-0.36343	1.777294	
Site3_1	1.056319	1.561978	1.461781	Saiden terrace 2
Site3_2	1.481419	1.89243	0.992481	
Site3_3	1.052711	1.434566	0.830707	
Site3_4	0.98506	1.544447	1.467601	
Site3_5	1.169658	1.644069	1.484053	
Site4_1	-3.36381	-3.19845	1.14745	Saiden lowland
Site4_2	-3.0546	-3.2524	0.935497	
Site4_3	-3.08844	-2.95914	0.682113	
Site4_4	-2.94968	-3.64096	0.303281	
Site4_5	-2.93273	-3.11341	0.377931	
Site5_1	-4.2216	1.967935	-0.20027	Kyrdemkulai lowland
Site5_2	-4.31987	1.595422	-0.81861	
Site5_3	-3.84443	2.070236	-1.5084	
Site5_4	-3.95068	1.981297	-0.56091	
Site5_5	-4.07288	1.559652	-0.40325	
Site6_1	-0.35808	0.833826	-0.45537	Kyrdemkulai upland terrace
Site6_2	-1.27444	0.724458	-0.3573	
Site6_3	-1.21215	0.772459	-0.34209	
Site6_4	-1.04122	0.999622	0.456633	
Site6_5	-1.14448	0.763328	0.330225	

Appendix 1.2 Principal component analysis performed using various fractions of C, N and P as defined variables in post-winter

A. Eigen values

Principal component	Eigen values	% variation	Cumulative % variation
PC1	9.41	49.5	49.5
PC2	3.91	20.6	70.1
PC2	2.33	12.3	82.4

B. Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variables	PC 1	PC2	PC3
pH_W	0.031	0.069	0.233
MBC_W	-0.280	0.195	0.140
TOC_W	-0.276	0.218	-0.097
DOC_W	-0.296	-0.178	0.129
BAS_W	-0.042	-0.016	0.506
SIR_1W	-0.284	-0.144	0.097
SIR_2W	-0.228	-0.284	0.083
SIR_3W	-0.273	-0.018	0.307
MBN_W	-0.277	0.241	0.101
Avl N_W	-0.105	-0.161	-0.385
TN_W	-0.270	-0.159	0.157
EON_W	-0.226	0.307	-0.148
pMN_W	-0.106	-0.427	-0.074
TP_W	-0.288	-0.192	0.025
AP_W	-0.136	-0.418	-0.082
MBP_W	-0.001	-0.268	0.397
DHA_W	-0.243	-0.228	-0.289
PHA_W	-0.297	0.104	-0.198

[TOC - total organic carbon, DOC - dissolved organic carbon, MBC - microbial biomass carbon, BAS - basal respiration, SIR – Substrate induced respiration, TN – total nitrogen, Avl-N – available nitrogen, MBN – microbial biomasss nitrogen, EN – extractable organic nitrogen, pMN – potentially mineralizable nitrogen, TP – total phosphorous, Avl-P – available phosphorous, MBP – microbial biomasss phosphorous, DHA – dehydrogenase activity, PHA – phosphatase activity. W denotes post-winter]

C. ***PCA scores***

Sites	Score 1	Score 2	Score 3	Name
Site1_1	2.92515	-1.99757	-1.51683	Saiden slope land
Site1_2	2.832072	-1.98112	-1.58519	
Site1_3	3.019891	-1.50086	-1.80805	
Site1_4	3.405246	-1.60315	-1.07437	
Site1_5	2.863815	-1.59535	-1.51727	
Site2_1	1.877339	0.010722	1.221824	Saiden upland terrace 1
Site2_2	2.12955	0.081435	0.962994	
Site2_3	2.365843	0.39624	1.459737	
Site2_4	2.553067	0.650608	1.26489	
Site2_5	2.415165	0.400198	0.967931	
Site3_1	0.920652	0.791982	2.89242	Saiden terrace 2
Site3_2	1.060575	0.807413	2.553056	
Site3_3	0.792607	0.482586	2.339359	
Site3_4	0.966828	0.024272	2.461989	
Site3_5	0.714736	0.724547	2.574273	
Site4_1	-5.76916	-1.90922	0.065174	Saiden lowland
Site4_2	-6.26714	-2.38914	0.36683	
Site4_3	-5.11821	-2.37606	0.244094	
Site4_4	-5.6571	-2.49839	0.065258	
Site4_5	-5.57106	-1.97941	0.364725	
Site5_1	-1.76903	3.820977	-0.8903	Kyrdemkulai lowland
Site5_2	-2.23483	3.848482	-1.39475	
Site5_3	-2.36985	3.348001	-1.1862	
Site5_4	-2.16622	3.556111	-1.4633	
Site5_5	-1.98346	3.887749	-1.41248	
Site6_1	1.907344	-0.65225	-1.06745	Kyrdemkulai upland terrace
Site6_2	1.58572	-0.53407	-1.36875	
Site6_3	1.524996	-0.52733	-0.88924	
Site6_4	1.497909	-0.6594	-1.2884	
Site6_5	1.547562	-0.62799	-1.34198	