Factors Regulating Methane Production And Emission From Natural Wetlands

Thesis

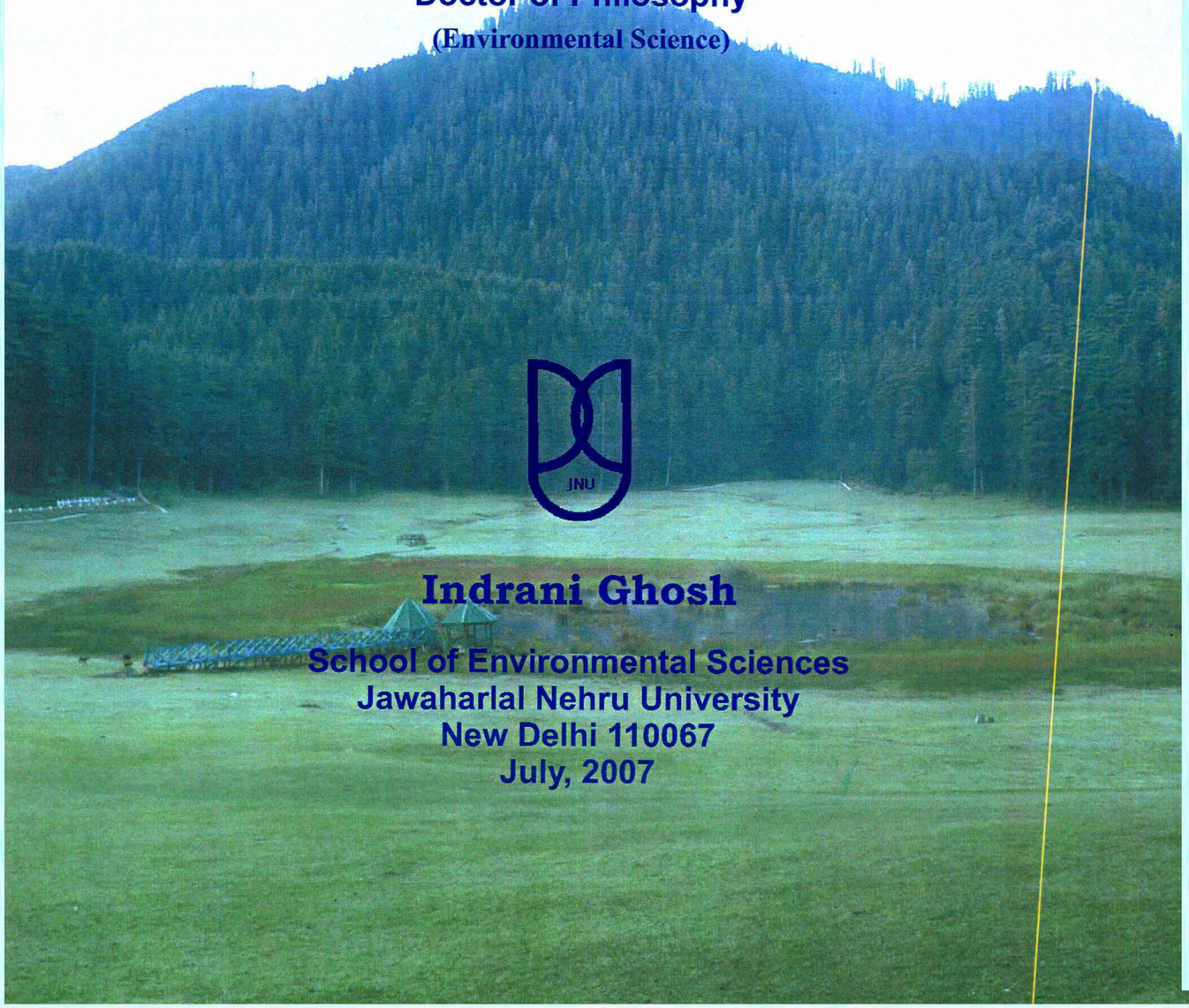
Submitted to

JAWAHARLAL NEHRU UNIVERSITY

for the award of degree of

Doctor of Philosophy

(Environmental Science)



Indrani Ghosh

School of Environmental Sciences
Jawaharlal Nehru University
New Delhi 110067

July, 2007

जवाहरलाल नेहरू विश्वविद्यालय
Jawaharlal Nehru University
SCHOOL OF ENVIRONMENTAL SCIENCES
New Delhi-110067

CERTIFICATE

30th July, 2007

The research work embodied in the thesis entitled ***"Factors regulating Methane Production and Emission from Natural Wetlands"*** has been carried out in the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. This work is original and has not been submitted in part or full for the award of any other degree or diploma in any other University or institution in country or abroad.

Indrani Ghosh

Prof. V.K. Jain
(Dean)

Prof. Brij Gopal
(Supervisor)

P.K.Gupta
(Scientist F)
(Co-Supervisor)

Gram : JAYENU TEL : 26717676, 26717557 SES : 2670 4302, 4303, 4304
FAX : 91-26162276

Asato Maa Sadgamaya,
Tamso Maa Jyotirgamaya,
Mrityor Maa Amritogamaya.

O Lord, Lead me from the Untruth to Truth,
Ignorance to Enlightenment,
Death to Immortality.

Verses from Brihadaranyaka Upanishad.

Dedicated to
Baba and Maa

Acknowledgement

O' Lord I am grateful to you for helping me to fulfill the aspiration of my parents.

*I take this opportunity to express my deep and sincere gratitude to my supervisor **Prof. Brij Gopal**. His sagacity, constant inspiration, encouragement, guidance and crack - of - the- whip at the last hour helped me complete my work.*

*My sincere acknowledgement to my co-supervisor, **Scientist (F) P.K. Gupta** for his support and valuable suggestions.*

*I sincerely express gratitude to **Prof. Dr. Peter Frenzel** for giving me the opportunity to work with him.*

*My sincere thanks to The Dean of the School of Environmental Sciences **Prof. V.K. Jain** for his co-operation and moral support.*

*I wish to extend my thanks to the staff (**Dr. P.D. Gaikwad, Mr. B.D. Sharma, R. Bhardwaj**) of Central Instrumentation Facility, S.E.S.*

*I sincerely owe my thanks to Mr. S. **Venkatesan** for his technical support.*

*I especially thanks **Mr. S. D. S. Rawat** and the other members of the office for their cooperation in completion of my work.*

I am greatly indebted to the teachers who taught me right from the nursery to my M.Phil days. Thanks to all of u for helping me make this journey so far.....

*This work would have been difficult without the support of **Gajender ji, Rawat ji, Nagender ji, Baburam, Anjum** and **Paramlal**.*

I take this opportunity to thank the funding agencies CSIR and DAAD (GAES) for supporting my research work.

*My warm acknowledgement to my relatives (didi, jamaibabu and others) and lab mates (**Narendra, Alefyah, Devlina, Rina**) for their moral and technical support.*

*Friends are the pillars of my strength. Thanks friends for being with me in fair as well as bad weather (**Karuna, Rita**, (tussi dono da jawab nahi) **Arindam, Anasuya, Dipanjan, Soma, Sarmistha, Purnima, Shashi, Dr. Sudipto Bhattacharya, Sandeep** and **Aiyaz Bhai**).*

*Thank you Doctor (**Dr. Sudipto Das**) for keeping me alive.*

*My neighbours envy and owners pride - **Hafeez**, Thanks.*

*Thanks my adorable sister **Mitrani Ghosh**. You are a precious gift in my life.*

*I owe my parents **Archana Ghosh** and **Sridhar Ghosh** much of what I am today. I thank them for their love, their support, patience and the confidence they bestowed on me.*

Indrani Ghosh

Abbreviations

μm	micrometer
ppbv	parts per billion by volume
mg $m^{-2} hr^{-1}$	milligram per meter square per hour
μg $m^{-2} hr^{-1}$	microgram per meter square per hour
gDW m^{-2}	gram Dry Weight per meter square
μg $gDW^{-1} hr^{-1}$	microgram gram Dry Weight per meter square
μmol $gDW^{-1}day^{-1}$	micromol per gram Dry Weight per day
Tg CH_4 yr^{-1}	Terragram methane per year
μM	micromol
H_2/CO_2	Hydrogen/Carbon-Dioxide
CO_2	Carbon Dioxide
H_2	Hydrogen
CH_3F	Methyl Fluoride
O_2	Oxygen
N_2O	Nitrous Oxide
H_2S	Hydrogen Sulfide
NO_3^-	Nitrate
Fe^{3+}	Iron (3+)
SO_4^{2-}	Sulfate (2-)
Fe (II)	Iron (II)
Mn (II)	Manganese
a.s.l	above sea level
RI	Respiratory Index
NEP	Net Ecosystem Production
NEE	Net Ecosystem Exchange
Q_{10}	Temperature Coefficient
$\Delta G°$	Gibbs Free Energy
pH	potentia hydrogenii
K_M	Michaelis-Menten Rate Constant
DGGE	Denaturing Gradient Gel Electrophoresis
T-RFLP	Terminal-restriction fragment length polymorphism
FISH	Fluoroscence in-situ hybridization
DNA	Deoxyribonucleic acid
G + C content	Guanine + Cytosine content
16S rRNA	16 S Ribosomal Ribonucleic acid
PCR	Polymerase chain reaction
TAE buffer	Tris acetate and EDTA (Sodium)
bp	base pair

List of Figures

Fig. 1 Schematic diagram showing degradation of organic matter

Fig.2A Location of Sampling Sites in India: Lake Khajjiar in Himachal Pradesh , Lake Bhalswa in Delhi.

Fig.2B Sites at Lake Khajjiar

Fig.3 Diagram of a chamber

Fig.4 Parts of *Scirpus* plant

Fig.5 Variation of Readily Mineralizable Carbon ($\mu g\ g^{-1}$), Microbial biomass ($\mu g\ g^{-1}$) and organic matter (%) in different depths and sites in Khajjiar Lake in 2005.

Fig.6 The variation in the average rate of methane emission (mg m^{-2} hr^{-1}) from unvegetated and vegetated soil at three different sites of Lake Khajjiar.

Fig.7 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to plant biomass (gDW m^{-2}) at Site-1.

Fig.8 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to total shoot length (cm m^{-2}) in Site-1.

Fig.9 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to plant biomass (gDW m^{-2}) in Site-2.

Fig.10 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to total shoot length (cm m^{-2}) at Site-2.

Fig. 11 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to plant biomass (gDW m^{-2}) at Site-3.

Fig.12 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to plant shoots (cm m^{-2}) at Site-3.

Fig.13 The variation in organic carbon content (%) of Bhalswa, Sandy and Garden soil over a period of four months.

Fig.14. Variation in rate of methane emission (mg m^{-2} hr^{-1}) for every half an hour interval, from bare and vegetated Bhalswa soil a) on 17 May 2006, b) 9 and 10 June 2006, c) 2 July 2006 and d) 5 October 2006

Fig.15 Variation in rate of methane emission ($\mu g\ m^{-2}\ hr^{-1}$) for every half hour an interval, from bare and vegetated Sandy soil on a) 10 and 11 May 2006, b) 14 June 2006, c) 1 July 2006 and d) on 10 October 2006

Fig.16. Variation in rate of methane emission (mg m^{-2} hr^{-1}) for every half an hour interval from bare and vegetated garden soil on a) 5^{th} and 6^{th} June 2006, b) 7^{th} July 2006, c) 12^{th} October 2006

Fig.17. Mean diurnal rate of methane emission from vegetated and unvegetated soil.

Fig.18. Comparison of the mean daily rate of methane emission from bare soil for over four months.

Fig.19. Comparison of the mean daily rate of methane emission from vegetated soil for over four months.

Fig.20. Relationship between organic carbon (%) and total methane emission (mg m^{-2})

Fig.21. The variation in the plant biomass (g m^{-2}) grown in Bhalswa, Sandy and Garden soil over the months.

Fig.22. Variation in daily rate of methane emission (mg m^{-2} hr^{-1}) against plant biomass (g m^{-2}) from a) Bhalswa, b) Sandy and c) Garden soil

Fig.23. Variation in the rate of methane emission (mg m^{-2} hr^{-1}) in different water levels

Fig.24 Variation in the rate of methane production ($\mu mol\ g^{-1}DW\ day^{-1}$) from soil of three different depths a) without and b) with CH_3F at Site-A with the rise in temperature.

Fig.25. The variation in the rate of carbon dioxide production ($\mu mol\ g^{-1}DW\ day^{-1}$) from soil of three different depths a) without and b) with CH_3F at Site-A with the rise of temperature. Respiratory Index is represented in open symbols. Fig.25

Contribution of methane produced by hydrogenotrophic methanogenesis with the rise of temperature at a) Site A and b) Site B

Fig.26. Steady state concentration of hydrogen gas (ppmv) from soil of three different depths a) without and b) with CH_3F at different temperatures at Site-A

Fig. 27. The variation in the rate of methane production (μmol $g^{-1}DWday^{-1}$) with the rise of temperature at three different soil layers a) without and b) with CH_3F at Site-B

Fig.28. The variation in the rate of carbon dioxide production (μmol $g^{-1}DWday^{-1}$)with the rise of temperature at three different soil layers a) without and b) with CH_3F at Site-B. Respiratory Index is also represented by open symbols.

Fig.29. Steady state concentration of hydrogen gas of a) without and b) with CH_3F of the three soil layers at different temperatures at Site-B

Fig.30 Contribution of methane produced by hydrogenotrophic methanogenesis with the rise of temperature at a) Site A and b) Site B

Fig. 31. Transient accumulation of a) acetate and b) propionate (μmol $g^{-1}DW\ day^{-1}$) in the soil of three different depth incubated with CH_3F at Site A

Fig.32 Transient accumulation of a) acetate and b) propionate (μmol $g^{-1}DW\ day^{-1}$) in the soil from three different depths incubated with CH_3F at Site B.

Fig.33 The variation in methane produced by acetoclastic pathway and acetate accumulation in the three soil layers at a) Site A and b) Site B

Fig.34. Variation in the relative fluorescence (%) of base pairs before incubation from a) 0-10 cm, b) 10-15 cm and c) 15-20 cm layer of soil at Site-A

Fig.35 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil samples of 0-10 cm layer incubated at different temperatures at Site A

Fig.36 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 0-10cm layer incubated with methyl fluoride at different temperatures of Site A.

Fig. 37 Variation in the relative fluorescence (%) of base pairs before incubation from a) 0-10 cm, b) 10-15 cm and c) 15-20cm layer of soil at Site-B

Fig. 38 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 0-10cm layer incubated at different temperatures at Site B

Fig.39 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 0-10cm layer incubated with methyl fluoride at different temperatures at Site B

Fig. 40 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 10-15cm layer incubated at different temperatures at Site B.

Fig.41 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil with methyl fluoride of 10-15cm layer incubated at different temperatures at Site B

Fig.42 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 15-20cm layer incubated at different temperatures of Site B

Fig.43 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 15-20 cm layer incubated with methyl fluoride at different temperatures of Site B

Fig.44 The variation in the rate of methane production (μmol $gDW^{-1}day^{-1}$) on incubation with or without external competitors for substrates used by methanogens from the a) 0-5 cm b) 5-15cm and c) 15-25cm of soil layers at three sites at Lake Khajjiar.

Fig.45 The variation in the rate of methane production (μmol $g^{-1}DW\ day^{-1}$) on incubation with or without external substrates used by methanogens from the three soil depths at three sites at Lake Khajjiar.

Fig.46 The variation in the rate of methane production (μmol $g^{-1}DW\ day^{-1}$) in relation to organic matter (%)from three depths of the three sites.

Fig.47 The variation in the rate of methane production in relation to microbial carbon and biomass from three depths of the three sites.

Fig.48. Rate of methane production at four different temperatures from a) Bhalswa soil, b) Sandy soil and c) Garden soil
Fig.49. The variation in the rate of methane production (μmol g^{-1}DW day^{-1}) on incubation with or without external competitors for substrates used by methanogens from soils at Lake Bhalswa
Fig.50. The variation in the rate of methane production (μmol g^{-1}DW day^{-1}) on incubation with or without external substrates used by methanogens from soils at Lake Bhalswa.

List of Tables

Table 1. Estimated sources and sinks of methane

Table.2 The variation in pH and conductivity in soils at the three sites of Lake Khajjiar in 2005

Table 3. The characteristics of the plants sampled from Site-1 at Lake Khajjiar.

Table 4. The characteristics of the plants sampled from Site-2 at Lake Khajjiar

Table 5: The characteristics of the plants that were sampled from Site-2 at Lake Khajjiar.

Table 6. Texture analysis of soils used for growing *Scirpus* plants.

Table 7. The variation in organic carbon (%), Total organic matter (%) and C/N ratio in three soil types in July

Table 8. Total methane emission (mgm^{-2}) from vegetated and bare soil in different month.

Table 9. Oxidation of methane by parts of plants

Table.10. Organic matter content (%) of soil from the two sites at Lake Khajjiar.

Table 11. Rate of methane and carbon dioxide production from soil of 0-10, 10-15, 15-20 cm layers at Site-C

Table 12. Predicted Terminal Restriction fragment (T-RF) length of archaeal 16S rRNA gene sequences from clone libraries and their phylogenetic affiliation

CHAPTER I

INTRODUCTION **1-4**

Aim and Objectives 4

CHAPTER II

REVIEW OF LITERATURE **5-28**

Wetlands 5
Methane 6
Sources and Sinks 6
Methanogens 8
Methanogenesis 9
Pathways of Methanogenesis 10
Factors influencing Methanogenesis 11
Hydrology 11
Electron acceptors 12
Organic Matter Content 13
Temperature 14
pH 15
Phylogenetic identification of methanogens using molecular techniques 15
Methane Production in Wetland Ecosystem 17
Methanotrophs 18
Methane Oxidation in wetlands 19
Methane Emission from Wetlands 20
Role of Plants 20
Mechanism of gas transport 21
Influence of Plant on Methane Production 21
Root exudates 21
Influence of Plant on Methane Emission 23
Species Composition 23
Biomass & Productivity 23
Stomata 24
Environmental Factors 24
Light 24
Temperature 25
Studies on methane emission from Wetlands in Asia 26

CHAPTER III

MATERIAL AND METHODS **29-46**

Sampling Sites 29
Collection of Soil 32
Sampling of peat at Lake Khajjiar in June, 2004 32
Sampling of peat at Lake Khajjiar in August, 2005 32
Sampling of mineral soil at Lake Bhalswa in January, 2006 32

Procedure of collection of methane samples from field 33
Chamber Design 34
Flux Calculation 35
Procedure of methane sampling under laboratory condition 36
Analysis 36
Analysis of gas samples 36
Analysis of soil samples 37
Organic Matter Content 37
Fatty acid analysis 37
Estimation of Total Nitrogen 37
Estimation of Total Phosphorus 38
Estimation of Nitrate (Inorganic Form): 38
Estimation of Nitrite (Inorganic Form): 39
Estimation of Microbial Biomass Carbon 39
Estimation of Total Organic Carbon 39
Estimation of Soil Texture 40
Soil Temperature 40
Molecular analysis of methanogens 40
DNA Extraction and PCR amplification 40
T –RFLP 41
Analysis of T-RFLP Profiles 41
Estimation of Biomass of plants 41
Study of methane emission from Lake Khajjiar 42
Methane Emission as influenced by different soil types planted with *Scirpus* sp. 43
Methane oxidation under laboratory condition 44
Study of the response of methanogens in presence of different alternate electron acceptors and substrates 45
Study of methane production potential at different temperature under laboratory condition 45
Study of methane production potential from soil using methanogenesis inhibitors 46
Statistical analysis 46

CHAPTER IV

RESULTS **47-109**

Methane Emission from Natural Wetland containing Peat Soil 47
Physicochemical characteristics of soil at Lake Khajjiar in 2005 47
Methane Emission from dominant vegetation at Lake Khajjiar 48

Methane Emission and Plant biomass 50
Methane Emission from Natural Wetland containing Mineral Soil 56
Physicochemical factors of Soil 56
Methane emission from vegetated and bare soil of Bhalswa Lake 58
Methane emission from vegetated and bare Sandy Soil 62
Methane Emission from vegetated and bare Garden Soil 67
Total Methane Emission 70
Mean Diurnal Methane Emission 71
Total Organic Carbon and Total Methane emission 73
Plant Biomass 74
Plant biomass and Methane Emission 75
Influence of water level on methane emission 76
Oxidation of methane 77
Methane Production from A Natural Wetland containing Peat Soil 78
Characteristic of the Peat collected from Lake Khajjiar in 2004 78
Methane and Carbon dioxide production from soils at Site-C 78
Rate of methane, carbon-dioxide and hydrogen production at different temperatures from Site-A and B 79
Site-A 79
Site B 83
Hydrogenotrophic Methanogenesis at Site-A and B 87
Fatty Acid Analysis 87
Structure of the Archaeal Community 92
Site-A 92
Site B 95
The response to the addition of competitors and substrates used by methanogens on methane production in Khajjiar Soil 103
Methane Production from Natural Wetland containing Mineral Soils 107
The response to the addition of competitors and substrates used by methanogens on methane production in Bhalswa Soil 108

CHAPTER V

DISCUSSION **110-126**

Methane Emission from Natural Wetlands 110
Role of plants in methane emission 110
Methane Emission and Soil Types 114
Methane Emission and Plant growth 115
Methane Emission and Water Depth 116
Methane Production at different temperatures from Natural Wetlands 117
Dominant pathway of methane production 119
Methanogenic precursors 122
Characterization of methanogens present in the soils of Lake Khajjiar 123
The response of methanogens to the presence of alternate electron acceptors 125

CHAPTER VI

CONCLUSION 127-129

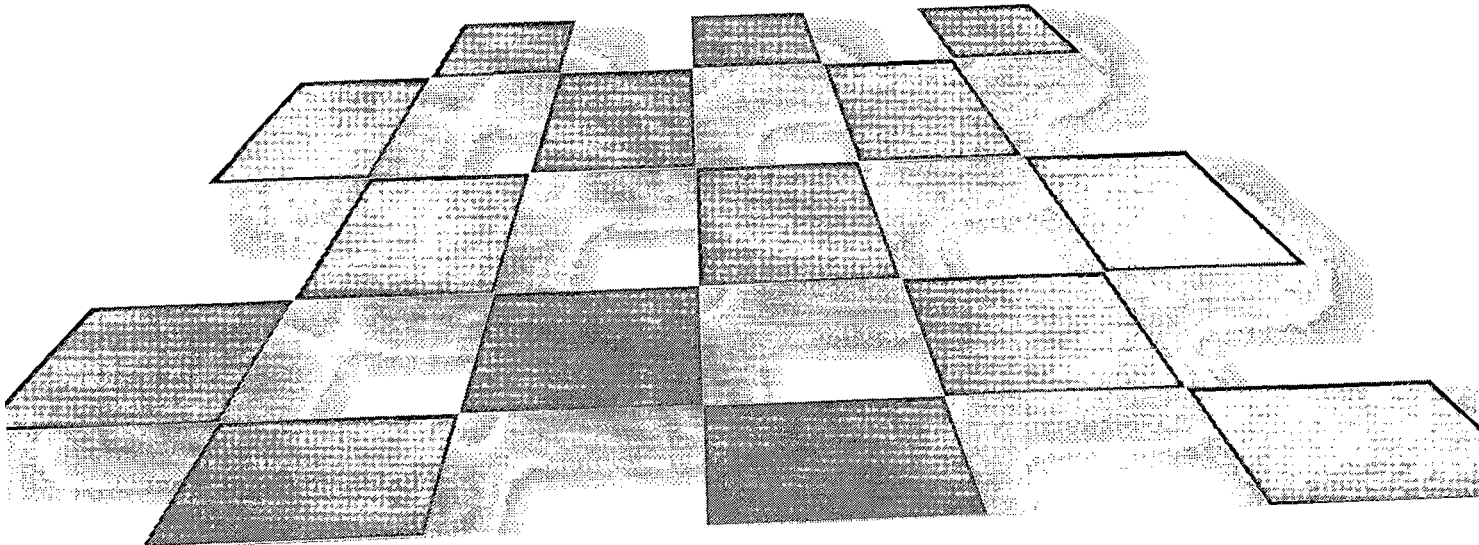
CHAPTER VII

SUMMARY 130-138
The role of plants in methane emission 133
Influence of edaphic and hydrological conditions on methane emission 134
Influence of temperature on methane production 135
Identification of dominant pathways of methane production in two natural wetlands 136
The response of methanogens in the presence of alternate electron acceptors 137

CHAPTER VIII

REFERENCES 139-149

INTRODUCTION



Chapter I

INTRODUCTION

Wetlands are distinguished by the presence of water, either at the surface or within the root zone. They are characterized by the presence of hydric soil and support hydrophytes that are adapted to this unique environment. The hydrology of a wetland creates the unique physicochemical condition that makes it a perfect niche for methanogens; forming a major source of methane gas (Mitsch and Gosselink, 2000). Methane is a greenhouse gas that contributes to global warming (IPCC, 1994). Wetlands contribute nearly 20% of the biologically produced methane (Watson et al., 1992).

Wetland have been classified by several authors on the basis of either their location (Cowardin et al., 1979), geomorphology and hydrology (Brinson, 1993a; b) or considering several environmental factors (Gopal et al., 1990). On the basis of environmental factors such as water regime, nutrient supply, main plant forms, and sediments, wetlands could be mires or peatlands (bogs, fens. swamps), temporary wetlands (temporary lakes and marshes) and water bodies (permanent lakes) (Gopal et al., 1990). Wetland soils could be characterized on the basis of their organic matter content. Mineral soils are poor in organic matter (less than 35 % on a dry weight basis) content and found in rice fields whereas organic soils are rich in organic matter (more than 35 % on a dry weight basis) and generally found in peatlands. Quantitatively half of the organic matter in a soil is composed of organic carbon that forms the major substrate for methanogens (Mitsch and Gosselink, 2000).

In wetlands the submergence of land initiates a chain of reactions wherein a consortium of different species of microbes act upon the available substrates one after another or together in the order of their potential to utilize hydrogen ions (DeLaune et al., 1983; Lovely and Klug, 1986; Bartlett et al., 1987; Lovely and Phillips, 1986; Burdige, 1993; Roden and Wetzel, 1996). According to sequential oxidation - reduction order, molecular O_2 is the first to be reduced at an Eh of about +30 mV followed by NO_3^- and Mn^{4+} at 250 mV, Fe^{3+} at + 125 mV and SO_4^{2-} at - 150 mV. Subsequent to SO_4^{2-} reduction, methanogens will start producing methane at –150mV

(Jakobsen et al., 1981; Patrick and Reddy, 1978; Ponnamperuma, 1972). This interaction is complex wherein the microbes have a competitive as well as symbiotic interaction.

Methanogens play a key role in the terminal step of C mineralization (Woese et al., 1978). Methanogens are basically C1 users and they are grouped according to their preference of substrate usage. They are either hydrogenotrophic (H_2/CO_2) or acetoclastic (acetate) depending upon the substrate they mineralize (Kelly & Chynoweth, 1981; Zehnder, 1978; Schink, 1992 Thauer, 1998). Studies show that these groups of methanogens vary in different wetlands and in different depths too (Popp et al., 1999; Svensson, 1984; Whiticar et al., 1986; Avery Jr. et al., 2002; Lansdown et al., 1992; Horn et al., 2003).

As any other life process, methanogenic activity is also influenced by the change in temperature (Svensson, 1984; Moore and Knowles, 1987; Crill et al., 1988). Earlier studies have shown the optimum temperature of potential methane production to be far higher than the in-situ temperature (Zeikus and Winfrey, 1976; Schulz et al., 1997; Avery Jr. et al., 2002).

Plants transport the gas produced in the anoxic soil through the interconnectd air-spaces in their tissues to the atmosphere. Different species of plants vary in their capacity to transport methane (van Veen et al., 1989; Whiting and Chanton, 1992; Minoda and Kimura, 1994). Plants of same species grown in different soil types may also vary in their capacity to emit methane. The probable underlying cause could be the variation in the methanogenic activity with the change in substrate quality and quantity corresponding to the change in soil types. Plants also influence the methanogenic activity antagonistically. The root exudates in the rhizospheric zone are rich in substrates that promote the methanogenic activity (Marschner, 1995; Kimura et al., 1997). At the same time oxygen is transported to the rhizosphere; which reduces the viability of the strictly anaerobic methanogens (Chanton and Dacey, 1991; Chanton et al., 1997; Watson et al., 1997; Frenzel, 2000).

Methane oxidation occurs in oxic zones like rhizospere, soil-water interface and even on aboveground parts of plant. Most estimates for the quantitative importance of methane oxidation have been studied in rice plants. In rice plants, the amount of methane oxidation is attributed to plant associated methane oxidation. Rates vary widely between 30 and 90% of total methane production (Banker et al., 1995; Bosse and Frenzel, 1997; Denier van der gon and Neue, 1996; Gilbert and Frenzel, 1995; Holzapfel-Pschorn et al., 1985,1986; Sass et al., 1990; Schutz et al., 1989).

The flooding of soil is a pre-requisite for methane production but methane emission from plants is also affected by the change in the water level suggesting that the water height above the soil surface and the overlying pressure also influences methane emission (Orawan and Suphasuk, 2002).

Thus, methane release from wetlands is controlled by several environmental parameters and is the net result of both production and consumption (Conrad, 1989). A detailed study to characterize the gas, its sources and sink as well as factors that govern the production and its escape to the atmosphere is a priority through out the world. In India, National Methane Campaign was set up at 1991 to quantify methane produced from the ricefields across the country (Parashar et al., 1996). Over the past few years studies on methane emission from natural wetlands such as mangroves (Purvaja and Ramesh, 2001; Purvaja et al., 2004) and estuaries (Verma et al., 2002) have been reported but the major focus still remains on ricefields. As methane production and emission from wetlands other than rice fields are poorly studied in the Indian subcontinent, the present study was undertaken in two natural wetlands differing in organic matter content. The occurrence of peat soil is limited to few pockets in the upper Himalayan region. Lake Khajjiar in Himachal Pradesh rich in peat soil represents the natural wetland containing higher organic matter. On the other hand, Lake Bhalswa on the outskirts of Delhi, is a wetland that contains mineral soil. The above wetlands were selected to study the factors regulating methane production and emission. The influence of factors like organic matter content, temperature, presence of electron acceptors on methane production was studied from soils at the above sites. The role of the dominant vegetation present at both the sites on methane emission was studied. In addition, the influence of different water depth and different organic matter content on methane emission from *Scirpus* plants was also estimated.

Aim and Objectives of the study

The specific aim and objectives of the study include:

I. Assess the role of dominant plants in methane emission present in Lake Khajjiar.

II. To investigate the diurnal rate of methane emission from *Scirpus* plants subjected to different hydrological and edaphic conditions

III. To investigate the effect of different nature of organic matter and soil on the rate of methane emission

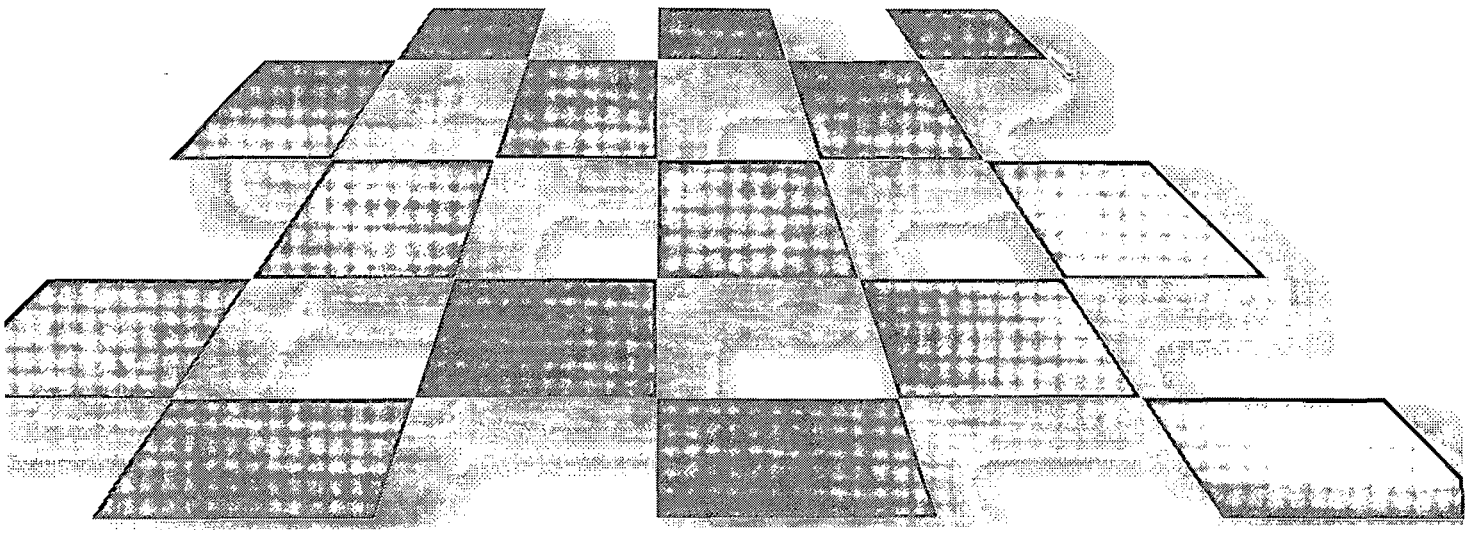
IV. To investigate the effect of water regime on methane emission

V. Determination of the influence of temperature on the rate of methane production from two natural wetlands

VI. Identification of the dominant pathway of methane production in two natural wetlands and molecular characterization of methanogens from Lake Khajjiar at different temperatures and soil depth

VII. Study the response of methanogens in the presence of alternate electron acceptors (added externally) from two natural wetlands

REVIEW OF LITERATURE



Chapter II

REVIEW OF LITERATURE

Wetlands

The interplay between land and water creates a unique ecosystem known as wetland. Cowardin et al., (1979) defined wetlands as

> *"lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water.... Wetlands must have one or more of the following three attributes: 1) at least periodically the land supports predominantly hydrophytes; 2) the substrate is predominantly hydric soil; and 3) the substrate is non-soil and is saturated with water or covered by shallow water at some time during the growing season of the year".*

Wetland soils, also known as hydric soils that are formed under a reduced condition are either organic or mineral soils. On the basis of the organic matter content, mineral soils are poor in organic matter (less than 35% on a dry weight basis) content whereas organic soils are rich in organic matter (more than 35 % on a dry weight basis). Mineral soils occur in wetlands such as in some freshwater marshes or riparian forests, they generally have a soil profile made up of horizons, or layers. Organic soils are applicable to many types of wetlands, particularly to northern peatlands; peat, a generic term for relatively undecomposed organic matter. Most peats contain about 40% organic carbon. Organic soils are different from mineral soils in several physiochemical features such as the former generally has acidic pH, low bulk density, high porosity, low to high hydraulic conductivity, high water holding capacity, often low nutrient availability, high cation exchange capacity. Comparatively, mineral soils usually have circumneutral pH, high bulk density, low porosity (40-55%) high hydraulic conductivity (except for clays), low water holding capacity, generally high nutrient availability, low cation exchange capacity dominated by major cations (Mitsch and Gosselink, 2000). Peatlands are characterized by an organic soil, but differ in hydrology, chemistry and, consequently, vegetation composition. High water levels and the consequent anoxia accompanied with low soil temperatures are considered the major causes for the imbalance between production and decay

resulting in being a storehouse of carbon (Clymo et al., 1998). As such the carbon sink of a peatland is labile, and sensitive to variations in environmental conditions (Alm et al., 1999, Bubbier et al., 2003 a, b). Quantitatively half of the organic matter in a soil is composed of organic carbon that forms the major substrate for methane production (Mitsch and Gosselink, 2000).

Methane

Methane is the most abundant organic species in the earth's atmosphere (Whalen, 1993) and ranks second to carbon dioxide in respect to global warming potential amongst other greenhouse gases. Methane absorbs infrared radiations originating from the Earth's surface, the atmosphere, and clouds in 7.7μm band. Thus, the heat that is trapped within the surface-troposphere system increases the temperature at Earth's surface, and in the troposphere (Ramanathan et al., 1985; Dickinson and Cicerone, 1986; Wang et al., 1976). Molecule for molecule, methane is 23 times more effective as greenhouse gas than carbon dioxide (IPCC, 2001).

The concentration of methane in the atmosphere presently stands about 1780 ppbv (Dlugokencky, 2001) compared to 650 ppbv during pre-industrial era (Pearman and Fraser, 1988). The rise is due to the rapid industrialization and the associated population growth during the last 200 years and links the increased concentration to anthropogenic activity. Once produced, methane resides in the atmosphere for 10-12 years (Pearman and Fraser, 1988). The interannual variability in the rise of methane over the past few years has ranged from 0 to 1 ppbv (Dlugokencky, 2001). The accumulation of methane in the atmosphere arises from an imbalance between the sources of the methane that increase the concentration and the methane sinks which lead to its destruction (Houghton et al., 1990).

Sources and Sinks

There are four major sources that contribute approximately equally to methane emissions: natural wetlands and tundra, rice fields, fossil fuel and ruminants. Other minor sources include biomass burning, landfills, termites, fresh water and ocean.
The main global sink of atmospheric methane is chemical oxidation by OH radicals in the troposphere that converts methane ultimately into carbon dioxide and water. These

products also contribute to greenhouse warming. Chemical oxidation accounts for about 85% or more of the global removal with the remainder, upto 10%, being removed by biological oxidation (Born et al., 1990).

Table1. Estimated sources and sinks of methane (Mosier et al., 2004).

Sources	**Tg CH_4 yr^{-1}**
Natural	
Wetlands	100-200
Termites	10-50
Oceans	5-20
Freshwater	1-25
CH_4 hydrate	0-5
Anthropogenic	
Coal mining, natural gas and pet industry	70-120
Rice paddies	20-150
Enteric fermentation	65-100
Animal wastes	10-30
Domestic sewage treatment	25
Land fills	20-70
Biomass burning	20-80
Sinks	
Atmospheric (tropospheric plus stratospheric)	
Removal	420-520
Removal by soils	15-45
Atmospheric increase	28-37

Wetlands account for about 20% of the total methane emission (IPCC, 2001). However, they vary considerably in their methane emission. Mathews and Fung, (1991) estimated that there are 5.3 million km^2 of wetlands in the world, of which 0.6 $\times 10^{12}$ km^2 are in temperate regions, 2.7 $\times 10^{12}$ km^2 in boreal Arctic and 2.0 $\times$ 10^{12} km^2 in tropical regions. Methane emission from these regions is 12, 32 and 71 Tg yr^{-1} respectively.

Methanogens

Methanogens are anaerobic microbes which reduce organic matter in the absence of other electron acceptors. The methanogens belong to the domain of Archeae (Woese, 1978) and are strictly anaerobes. Methanogens can be classified taxonomically according to morphology, motility, electron microscopy images, colony morphology, nutritional spectrum, growth rates, growth conditions, metabolic end-products, Gram staining, susceptibility to lysis, antigenic fingerprinting, lipid analysis, distribution of polyamines, nucleic acid hybridisation, G + C content of the DNA, 16S rRNA sequencing and sequence analysis (Boone and Whitman, 1988). According to the above criteria, five orders, ten families, twenty-six genera, and seventy-four valid species have been defined (Boone et al., 1993):

Order *Methanobacteriales* contains two families. Family *Methanobacteriaceae* has four morphologically distinct genera. They include species retrieved from a variety of habitats (freshwater, bovine rumen, wood, termite guts, etc), which are able to use substrates as H_2/CO_2, 2-propanol, formate, and methanol for CH_4 production. Family *Methanothermaceae* consists of one single genus of extreme thermophile hydrogenotrophs.

Order *Methanococcales* contains two families, *Methanococcaceae*, and *Methanocaldococcaceae*, and four genera of hydrogenotrophs from mainly marine and coastal environments. Most of the species are able to use both H_2 and formate as electron donors.

Order *Methanomicrobiales* comprises three families and nine genera of hydrogenotrophic methanogens. Family *Methanomicrobiaceae* contains species isolated from various environments (rumen, marine sediments, etc). Family *Methanocorpusculaceae* consists of three genera utilizing H_2/CO_2 and formate as substrate. Family *Methanospirillaceae* is a family with a single genus; members have been reported from various habitats, and are able to use different electron donors for methanogenesis from CO_2.

Order *Methanosarcinales* regroups all acetotrophic, and/or methylotrophic methanogens into two families. Family *Methanosarcinaceae* contains six genera of very versatile methanogens retrieved from various habitats, and able to use H_2/CO_2, acetate, or methyl compounds as substrate. Family *Methanosaetaceae* includes one genus of obligate acetotrophic methanogens.

Order *Methanopyrales* is a new order of hyperthermophilic methanogens, which are not related to any other known methanogens. The order comprises of one single family *Methanopyraceae* that has a single species *Methanopyrus kandleri*.

Methanogenesis

Methanogenesis is the process by which the methanogens act upon substrates to produce methane and subsequently derive energy to drive the processes of life.

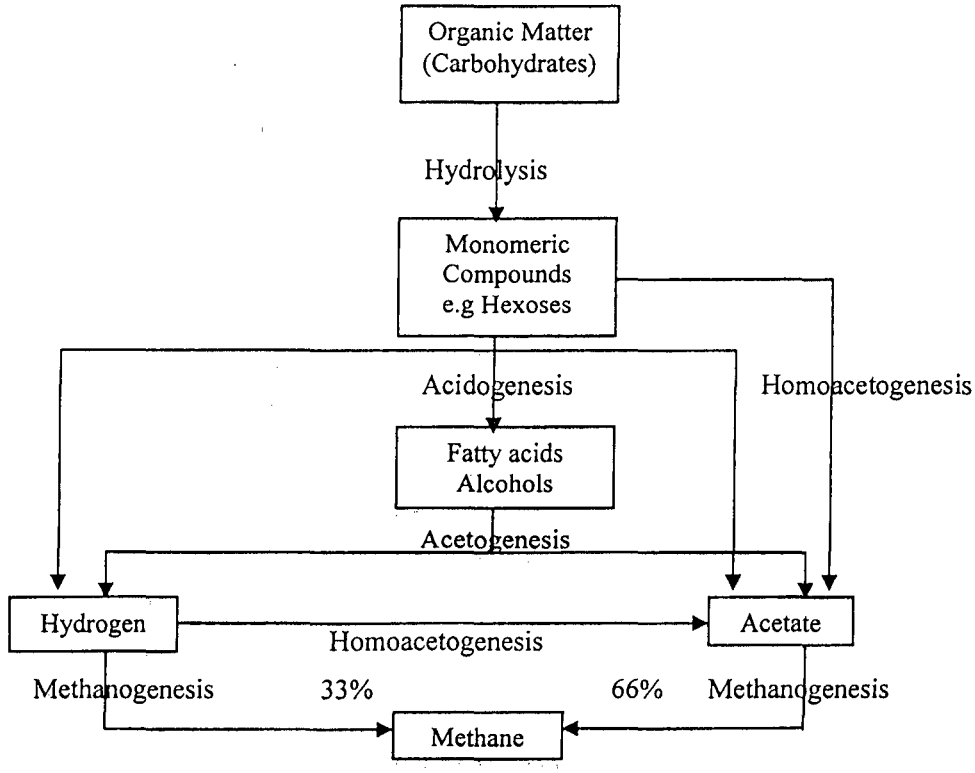


Fig.1 Schematic diagram showing degradation of organic matter and production of methane (*Adapted from: Conrad, 1999 and Garcia et al., 2000*)

In anaerobic environments glucose from polysaccharides is completely decomposed into CO_2 and CH_4.

Glucose----------$3CO_2+3CH_4$ $\Delta G°' = -418.1$ k J/mol

The reaction is not catalyzed by methanogens alone but by the syntrophic association of several groups of microorganisms (Thauer, 1998).

In natural anaerobic habitats which contain complex organic compounds and where light, sulfate, and nitrate are limiting, methanogens are linked to chemoheterotrophic bacteria for the degradation of organic substrates in a four-step process: (1) hydrolysis of polymers by hydrolytic microorganisms, (2) acidogenesis from simple organic compounds by fermentative bacteria, (3) acetogenesis from metabolites of fermentations by homoacetogenic or syntrophic bacteria, (4) methanogenesis from H_2+CO_2, acetate, simple methylated compounds or alcohols $+CO_2$ (Garcia et al., 2000) (Figure 1).

Pathways of Methanogenesis

The metabolic pathway of methane formation are the following:-

1) The Hydrogenotrophic or H_2 / CO_2 reducing pathway- in this the energy substrate is (electron donor) is H_2, formate, or certain alcohols, and the electron acceptor is CO_2, which is reduced to methane (Thauer et al., 1993). Thirty-eight species identified that are capable of exploiting the substrate using this pathway. This pathway is very important for maintaining the very low concentrations of H_2 and formate typical of the anaerobic habitats and facilitates the process of interspecies electron transfer. Hydrogenotrophic methanogens receive their substrate (H_2) directly from fermentative bacteria and interspecies transfers (Thiele and Zeikus, 1988; Lovley, 1985). This nutritional reaction is thermodynamically the more favourable, in terms of free energies ($\Delta G°'$), of those inducing methane synthesis (Garcia et al., 2000).

2) Methylotrophic pathway- the energy substrate is one of the varieties of methyl-containing C-1 compounds. In the absence of molecular hydrogen, cells are forced to oxidize some of the methyl groups for the regeneration of reduced electron carriers. Three molecules of methanol are reduced to methane, and a fourth molecule is oxidised to CO_2. The major difference between the hydrogenotrophic

and the methylotrophic pathway is the source of reducing equivalents for reductive reactions. Twenty species identified that are capable of exploiting the substrate using this pathway.

3) The Acetoclastic pathway- in this pathway the methyl carbon of acetate is reduced to methane and the carboxyl carbon is oxidized to CO_2. This is the most dominant pathway as maximum portion of methane in nature originates from the decomposition of acetate. Nine species identified that are capable of exploiting the substrate using this pathway.

All methanogenic pathways use two-electron reduction of Methyl-coenzyme M to methane catalyzed by Methyl-coenzyme M reductase. Redox reactions involved in the above processes are partly catalyzed by membrane- bound enzyme systems that generate, or in the case of endergonic reactions, use electrochemical ion gradient. The H_2: heterodisulfide oxidoreductase F420 H_2: heterodisulfide oxidoreductase and the CO: heterodisulfide oxidoreductase, are novel systems that generate a proton motive force by redox-potential –driven H+ translocation.

Factors influencing Methanogenesis

Methane production is influenced 1) by the aeration of the soil, as methanogens require anoxic condition to produce methane, 2) by the presence of the electron acceptors, like nitrate and sulfate, 3) by type and amount of available organic matter 4) by the size of the methanogenic population and 5) temperature.

The interaction of the environmental factors influencing methane production has been studied in in-situ condition as well as in micocosms.

Hydrology-The height of the water table is an important factor controlling methane fluxes and emissions from wetlands, because it controls the extent of anaerobic-methane producing soil and the amount of methane consumed before it escapes (Bubier, 1995; Sundh et al., 1992, 1994; Bubier and Moore, 1994). Methane fluxes can vary significantly depending on whether the water table is rising or falling. Dise et al., (1993) observed that water table position controlled 62% of the variance from a range of peat land ecosystems in Northern Minnesota. Rates of methane production

were lower for low water table and high for high water table (Gruenfield and Brix, 1999). When waterlogging is reduced by lowering the water table, wetland soils begin to exhibit characteristics which resemble those of other ecosystems: Methane and DOC release are suppressed, while mobilisation of the store of carbon as CO_2 and the release of sequestered inorganic nutrients increase (Moore and Knowles 1989; Heathwaite, 1990; Freeman et al., 1993; Freeman et al., 1994), oxygen availability increases, hence potential for aerobic degradation of organic matter and methane oxidation increases. Moore and Roulet, (1993), showed that methane fluxes could differ by more than 100 fold at a given water table height depending on whether the water table was rising or falling. In an ephemeral wetland that is periodically flooded, methane production has been observed to be possible even from rewetted floodplain environments that had earlier experienced an extended dry phase (Boon et al., 1997). With the increase in level of submergence methane emission was enhanced (Parashar et al., 1994; Adhya et al., 2000). Alternately flooded plot emits less methane compared to continuously flooded plots (Adhya et al., 2000). In deep waters, the deeper layers of the floodwater may also become anoxic during the crop cycle (rice plants) permitting methanogenesis from the large quantity of organic material available from rice culms, nodal roots and dead aquatic biomass (Whitton and Rother, 1988).

Electron acceptors- Methane production is influenced by the presence of alternative electron acceptors like O_2, NO_3^-, Fe^{3+} and SO_4^{2-} (Achtnich et al., 1995; Jakobsen et al., 1981). The presence of alternative electron acceptors leads to competition for the common electron donors, particularly H_2 and acetate, between bacteria using alternative electron acceptors and methanogens. Such competition effects can be described by thermodynamics, but also by kinetic parameters as given by the Michaelis-Menten equation. The affinity constant K_M of the kinetic theory is correlated to the Gibbs free energy under standard conditions (ΔG°) (In general, the more negative ΔG° the lower the K_M) (Bodegom and Stams, 1999). Inhibition effects can be caused by metabolic products and by redox effects. The production of the toxic intermediates during denitrification of nitrite, NO, N_2O was speculated to be responsible for the inhibition of methanogenesis, rather than the competition for acetate between methanogens and denitrfiers (Balderston and Payne, 1976; Klueber and Conrad, 1998; Roy and Conrad, 1999).

Carbon and electron flow are altered when sulfate is added to sediments. In the presence of sulfate, the methyl position of acetate is converted to CO_2.

Methanogens are usually poor competitors against other heterotrophic microorganisms for limiting amounts of substrate, so that CH_4 production normally commences after reservoirs of alternative electron acceptors are depleted (Achtnich and Rude, 1988; Capone and Kiene, 1988). As a consequence, activity of heterotrophic microorganisms other than methanogens may suppress CH_4 production rates in substrate limiting environments such as salt and brackish systems with high abundance of sulfate (DeLaune et al., 1983; Lovley et al., 1986; Middelburg et al. 1996) or Fe (III)-rich freshwater systems (Lovly and Phillips 1986; Burdige 1993; Boon and Mitchell, 1995). Recently, Roden and Wetzel (1996) demonstrated the significant suppression of CH_4 production in a vegetated freshwater wetland sediment by microbial Fe (III) reduction compared to a non-plant control sediment.

The production of the toxic intermediates during denitrification of nitrite, NO, N_2O was speculated to be responsible for the inhibition of methanogenesis, rather than the competition for acetate between methanogens and denitrfiers (Balderston and Payne, 1976; Klueber and Conrad, 1998; Roy and Conrad, 1999).

Organic Matter Content- Organic matter has been reported to be a good predictor of CH_4 production capacity in peatlands, with highest CH_4 emissions from organic matter that is relatively labile and lowest CH_4 emissions from organic matter that is relatively recalcitrant (Yavitt and Lang 1990). Crozier et al., (1995) and Yavitt and Lang (1990), found a significant relation between loss-on-ignition organic matter and CH_4 emissions. Schimel (1995) believes that soil organic matter is the main substrate for methanogens, while others believe that recently dead plant material is the main substrate (e.g. Chanton et al., 1995; Whiting and Chanton, 1993). Chanton et al., (1995) suggest that the main source of organic matter for methanogens is recently fixed organic compounds, most likely the dissolved organic compounds produced from the decay of recently produced litter, roots and root exudation products. In correspondence with this, van den pol-van Dasselaar et al., (1999) found a better relationship between dissolved organic carbon and CH_4 emissions than between loss-

on-ignition and CH_4 emissions at Brampjesgat (Netherlands). This factor is further reviewed in the influence of plants on methane production and oxidation.

Temperature-Methane production as any other life processes is influenced by temperature. Temperature has a major influence on microbial activities (Mac Donald et al., 1995). It is well documented that particular communities of microbes are well adapted to particular range of temperatures (Allen and Brock, 1968; Zogg et al., 1997). The temperature range in which methanogens are found is quite broad, from 4 to 110°C (Garcia et al., 2000). Soil slurries incubated at different ranges of temperature by different workers, from 0-45°C has shown that the maximum activity is around 25°C-35°C (Dunfield et al., 1993; Fey and Conrad, 2002). The rate of methane production increases exponentially with the increase of temperature (Mc Donald et al., 1998; Daulat and Clymo, 1987). MacDonald et al., (1995) found an apparent increase in the pool of labile C with an increase in soil temperature. One reason for the shift in activity with the shift in temperature could be due to shift in the use of the available substrate in the soil apart from the effect on biomass and enzyme activity (McCaugherty and Linkins, 1990). Zogg et al., (1997), found that an apparent increase in the pool size of labile carbon with increasing temperature was associated with changes in the composition of the microbial community as determined through molecular analysis. Soil microbes vary in their affinity for various substrates; therefore a change in the diversity of microbial activity may render additional compounds to the labile carbon pool (Andrews et al., 2000). Temperature induced changes in the composition or activity of microbial communities can modify the biochemical pathways of primary resource exploitation and the production of secondary material and can consequently affect the pool size of the resource that behaves as labile or recalcitrant. Berg et al., (1993) support the process of formation of more stable residual materials at more favourable temperature. Often it has been observed that in a system such as a lake, the optimum temperature at which there is maximum potential for methane production, is far higher than the maximum in-situ temperature (Zeikus and Winfrey, 1976; Schulz et al., 1997; Avery Jr. et al., 2002). In soils from rice field, hydrogenotrophic methanogenesis contributed to the major methane production with the rise of temperature as compared to acetoclastic methanogenesis. The effect of temperature was found to be less on the process of homoacetogenesis (Fey and Conrad, 2000).

pH- The pH of a soil has a minor influence on methane production. Most methanogens grow over a relatively narrow pH range (Garcia, 1990; Whitman et al., 1992, Boone et al., 1993) although in vivo methanogenesis studies have shown that it does occur in acidic environments such as peat bogs (Williams and Crawford, 1984)). *Methanobacterium espanolae*, a moderately acidophilic methanogen grows optimally at pH between 5.6 and 6.2 but is unable to grow and produce methane at pH 4.7 (Kotelnikova et al., 1993). Two alcaliphilic methanogenic members of genus Methanobacterium have a pH optimum between 8 and 9, whereas the two halophilic methylotrophic methanogens, *Methanolobus oregonensis* (Liu et al., 1990) and *Methanosalsus zhilinae* (Mathrani et al., 1988; Kevbrin et al., 1997), grow at pH 9.2. The pH sensitivity of methane production differed among Canadian peat soils. In the common neutral peats, methanogenesis was acid sensitive. In acid black hole sites, however methane production was balanced by low pH, indicating organisms were acid adapted (Valentine et al., 1994).

Yavitt et al., (2005) observed Bleak Lake Bog (with pH-3.9) and Marrol (with pH-3.6) had the high acidic pH values. There has been some debate (Dunfield et al., 1993) about the way low pH affects methanogens in peat. One is direct toxicity per se, similar to that for rumen methanogens (Van Kessel and Russell, 1996). The other is an indirect effect of acidity on H_2 production that subsequently fuels methane production, similar to that in lake sediments (Goodwin et al., 1998). Methanogens found to grow in very acidic or alkaline environment have a reduced activity compared to those growing in optimum pH.

Phylogenetic identification of methanogens using molecular techniques

The application of molecular markers to detect microorganism helped in targeting specific community, saved time and was more precise compared to different culturing and isolation techniques.

Ribosomal RNA's are functionally constant, universally distributed, and moderately well conserved in sequence across broad phylogenetic distances. The degree of similarity in ribosomal RNA sequences between two organisms indicates their relative relatedness. From comparative sequence analysis, molecular genealogies can be constructed leading to phylogenetic trees that show the most probable evolutionary

position of organism relative to one another. The ribosomal RNA could be distinguished into three molecules based on centrifugation-5S, 16S and 23S. 16S and 23S contain several regions of highly conserved sequence useful for obtaining proper sequence alignments; yet contain sufficient sequence variability in other regions of the molecule to serve as excellent phylogenetic chronometers. As 16S rRNA is experimentally more manageable, hence it is widely used to determine the phylogeny of the prokaryotes and the eukaryotes (Madigan et al., 2003).

Primers (a sequence of bases) for the study of methanogen populations have been designed to amplify various regions of the 16S rRNA. Some primers target the domain *Archaea* generally, other are specific for methanogenic 16S rRNA. The basic primers used to detect the methanogens target the a) the Archaeal 16S rRNA b) mcr gene of the methanogens, or c) the Methanogenic 16S rRNA. Most scientists employ the Polymerase Chain Reaction (PCR) to amplify directly the genes encoding 16S rRNA from genomic DNA and then process the purified product for various techniques like Denaturing Gradient Gel Electrophoresis (DGGE), Terminal-restriction fragment length polymorphism (T-RFLP), Fluoroscence in-situ hybridization (FISH).

T-RFLP analysis combined with phylogenetic analysis of cloned sequences of the archaeal 16S rRNA genes showed that the methanogenic community consisted mainly of *Methanomicrobiales* and *Methanosaetaceae*. The relative abundance of *Methanosaetaceae* decreased with depth, whereas that of *Methanomicrobiales* slightly increased. Hence, the vertical distribution of the functional characteristics (CH_4 production from acetate versus H_2/CO_2) was reflected in the structure of the community consisting of acetotrophic (*Methanosaetaceae*) versus hydrogenotrophic (*Methanomicrobiales*) phenotypes in Lake Dagow, Germany (Chan et al., 2005)

Cadillo-Quiroz et al., (2005) examined methanogenic activities and methanogen populations at different depths in two peatlands, McLean bog (MB) and Chicago bog (CB). Methanomicrobiales or Fen group was detected from the bog. The methanogen populations detected by T-RFLP in deeper portions of MB were mainly E2 and the uncultured euryarchaeal rice cluster (RC)-II group, whereas populations in the less acidic CB deep layers were considerably different, and included a *Methanomicrobiales* clade that was called as E1-E1.

In a mire from Finland, the T-RFLP analysis showed that most 16S rRNA gene sequences were affiliated with methanogens and all McrA sequences clustered with the exclusively hydrogenotrophic order *Methanobacteriales* (Metje and Frenzel, 2005).

The composition of the archaeal community was determined in the peat samples of West Siberian peat bog 'Bakchar' by T-RFLP analysis and sequencing of amplified SSU rRNA gene fragments. The study showed that members of *Methanomicrobiaceae*, *Methanosarcinaceae* and Rice cluster II (RC-II) were present. Other, presumably non-methanogenic archaeal clusters (group III, RC-IV, RC-V, RC-VI) were also detected. Fluorescent *in situ* hybridization (FISH) showed that the number of bacteria decreased (from 24 x 10^7 to 4 x 10^7 cells per gram peat) with depth (from 5 to 55 cm below the water table), whereas the numbers of *Archaea* slightly increased (from 1 x 10^7 to 2 x 10^7 cells per gram peat). *Methanosarcina* spp. accounted for about half of the archaeal cells (Kotsyurbenko et al., 2004).

Methane Production in Wetland Ecosystem

Acetate and hydrogen/carbon-dioxide are usually the immediate precursor of methanogenesis (Kelly and Chynoweth, 1981; Zehnder, 1978; Schink, 1992). In an ideal situation of methane production, hydrogen/carbon-dioxide should account for 33% of total methanogenesis (Conrad, 1999) but in the natural environment there is a lot of deviation.

There is a shift in the dominance of substrate utilization from acetate in the vegetated zone to CO_2 reduction in the unvegetated zones (Popp et al., 1999; Bellisario et al., 1999). This is primarily due to the influence of availability of fresh organic matter in the case of vegetated sites. Methanogenic community also varied with depth. In fen ecosystems, acetoclastic methanogenesis is often found to be predominant in upper peat layers (Galand et al., 2002; 2003). Input of labile carbon sources from the rhizosphere of vascular plants (e.g. *Carex* or *Eriophorum* sedges) promotes the acetotrophic pathway in upper minerotrophic layers. In the deeper fen layers, where mostly recalcitrant old peat is present, hydrogenotrophy is the dominating pathway (Kelley et al., 1992; Popp and Chanton, 1999). The stratification of the methanogens

with depth is explained on the basis of temperature difference in the in-situ condition as acetoclastic methanogens have lower temperature optima compared to hydrogenotrophic ones (Wagner and Pfeifer, 1997; Fey and Conrad, 2000). In most of the freshwater wetland environments (pH neutral), acetate is found to be the dominant substrate (Whiticar et al., 1986; Avery Jr et al., 2002) whereas in peat-accumulating wetlands, hydrogenotrophy is the dominant pathway for methanogenesis (Whiticar et al., 1986; Lansdown et al., 1992; Hornibrook et al., 1997; Horn et al., 2003). In bogs, vegetation is often restricted to non-aerenchymous plants (e. g. *Sphagnum* mosses), and hydrogenotrophy may be the dominant pathway at all depths (Horn et al., 2003). The association of peatland types with methanogenic pathways remains, however, difficult to establish. For instance, Avery et al., (1999a) suggested that pathways change with season (winter to summer) according to rates of primary production in Buck-Hollow bog (Michigan). Galand et al., (2005), observed methane production changed from hydrogenotrophy during winter to acetotrophy during late spring, following vegetation growth. Metje and Frenzel, (2005), found hydrogenotrophic methanogenesis to account for 80% of total methanogenesis in a mire in Northern Finland. Contrary to the belief that hydrogenotrophic methanogenesis contributes more in acidic bogs, Kotsyurbenko et al., (2004), found in an acidic West Siberian peat bog that acetoclastic and hydrogenotrophic methanogenesisis contributed to total methane production in 2:1 ratio at all incubated temperatures; 4, 15 and 25°C. Unfortunately, literature search reporting the character of methanogenic population using molecular marker from mineral soils of estuary or freshwater sediment did not yield any result.

Methanotrophs

Methanotrophs utilize methane as their sole source of carbon and energy. Methanotrophs are obligately aerobic-gram negative bacteria. They can be isolated from a wide variety of environments incuding soils, sediments and freshwater. The methanotrophs can be divided into three groups (Ritchie et al., 1997):

Type I methanotrophs include the genera *Methylomonas, Methylomicrobium* and *Methylobacter*. They assimilate formaldehyde produced from the oxidation of methane (via methanol) using the ribulose monophosphate pathway (Anthony, 1982; 1986).

Type II methanotrophs utilize the Serine pathway for formaldehyde assimilation, posses intracytoplasmic membranes arranged around the periphery of the cell, contain pre-dominantly 18-Carbon fatty acids and include the genera *Methylosinus* and *Methylocystis.*

Type X methanotrophs such as *Methylococcus capsulatus* appear to posses some properties of both Type I and II organisms.

Methane Oxidation in wetlands

In an oxic environment, methanotrophs utilise methane to produce carbon dioxide and water (Cicerone and Oremland, 1988). Methane oxidation can be carried out by high-affinity methane oxidizers in aerobic soils, like forest soil and agricultural soils, and by low-affinity methane oxidizers in anaerobic soils where methane is simultaneously produced (Bender and Conrad, 1993; 1994).

In wetlands methanotrophs are usually found in the oxic layers like soil-water interface, water-air interface, rhizosphere and aboveground surfaces of few plants. Methane oxidation in wetland soils is influenced by oxygen availability, which in turn depends on the level of water table. Dunfield et al., (1993), observed that temperature changes had lesser effect on methanotrophs compared to the methanogens from wetland soil. Changes in methane and oxygen concentrations during the growing season of the plant affect the methanotrophs (Svensson and Rosswall, 1984; Whiting and Chanton, 1993), which is reflected in the methane flux. Though few studies on *Eriophorum* species suggest that it does not support methane oxidation (King et al., 1990; Chanton et al., 1992 a,b; Kelkar and Chanton, 1997; Frenzel and Rudolph, 1998) and the plausible explanation offered is the difference in the quality and quantity of root exudates of the above plants (Frenzel and Rudolph, 1998). Aquatic plants enhance CH_4 emission rates by serving as gas conduits by means of aerenchyma by which the produced methane bypasses the oxidation zone (Bubier et al., 1993; Bubier and Moore, 1994; Thomas et al., 1996).

Methane Emission from Wetlands

Methane produced in the anoxic soil by the methanogens escapes to the atmosphere through diffusion which is a slow and a gradual process (Chanton et al., 1993; Happell et al., 1995). It is a function of surface water concentration of methane, wind speed and methane supply to the surface water (Sebacher et al., 1985). Methane is also released to the atmosphere in bulk when the accumulation of the gas below the soil surface creates a buoyant force greater than the overlying hydrostatic force, the process is known as ebullition (Yamamoto et al., 1976). This accounts for 64% (Bartlett et al., 1988) to 70% (Crill et al., 1988) of total flux.

Apart from the above two pathways, vascular plants with their aerenchyma cells (an adaptation for survival in the anoxic environment-Schulthorpe 1985; Koncalova, 1990; Armstrong et al., 1991a) act as a conduit and form a dominant pathway for the methane to escape to the atmosphere (Sebacher et al., 1985; Shannon and White, 1994; Van der Nat and Middelburg, 1998 a,b). This may merely not be a passive process; rather plants employ pressure-induced convective throughflow to transport the gas (Brix et al., 1992).

Role of Plants

Wetland plants play an important role in the transport of methane from the point of production to the atmosphere. Methane transport through wetland plants is often mentioned as the dominant pathway for methane release to the atmosphere (Schimel, 1995; Frenzel and Rudolph, 1998; King et al., 1998; Bellisario et al., 1999; Greenup et al., 2000).

The emission of methane by a vascular plant is a resultant of three process:-

1) the production of methane by methanogens in the anoxic sediment and supply to the roots
2) the oxidation of methane by methanotrophs present in the rhizosphere and on the plant surface
3) the capacity of plant to transport gas to the atmosphere from roots and rhizomes

Mechanism of gas transport

The gas transport in wetland plants is accomplished either by simple diffusion (Armstrong 1979; Sand Jensen and Prahl, 1982) or by pressurized convective flow (Armstrong et al., 1991a). Wetland species like *Oryza sativa* (Seiler, 1984; Denier van der gon and van Breemen, 1993), *Peltandra virginica*, *Carex* sp. (Popp et al., 1999), *Equisetum fluviatile* (Hyvoenen et al., 1998) and *Cladium jamaicense* employ a methane transport system based on molecular diffusion. The pressure, in the plants employing convective throughflow, is generated by gradients between the internal gas spaces and the surrounding atmosphere that is vented through rhizomes and old shoots to the atmosphere (Dacey, 1981a; Armstrong and Armstrong, 1991). Internal pressurization induced convective gas flows are important adaptations to growth in anoxic sediment (Brix et al., 1992). Internal pressurization in wetland plants can occur as a result of two physical processes; thermal transpiration and humidity-induced pressurization. Both require a porous partition within the plant tissue, ideally with pore sizes in the Knudsen regime i.e less than the mean free diffusive path length of the gas molecules (<0.1μm), and a consumption of energy in the form of heat. In addition, humidity induced pressurization requires a constant supply of water inside the plant. Thermal transpiration is the movement of gases through a porous partition when there is a gradient in temperature across the partition. Thermal transpiration leads to a pressure gradient across the partition, the pressure being higher on the warmer side (Brix et al., 1992). Gas transport in *Typha latifolia, T.angustifolia, Nymphea odorata, Nuphar luteum, Nelumbo nucifera, Nymphoides pelata and Phragmites australis* is dominated by convective throughflow transport in daylight (Armstrong and Armstrong, 1991; Bendix et al., 1994; Brix et al., 1992; Dacey, 1981a; b; Grosse and Mevi-Schutz, 1987; Tornbjerg et al., 1994). At night when these pressure gradients are absent molecular diffusion is the primary transport mechanism.

Influence of Plant on Methane Production

Root exudates-Primary production of plants forms the main carbon input to the soil (Wardle, 1999; Kuzyakov and Domanski, 2000). Two different forms of carbon input by plants into the soil can be distinguished. Living plants release root exudates that mainly comprise of easily degradable organic compounds including carbohydrates (60-70%), N free carbonic acids (20-35%) and amino acids (2-25%) (Kraffczyk et al., 1984; Jones, 1998; Gransee and Whittenmayer, 2000) and dead plant tissues enter the

soil food web by decomposition of leaf- and root-litter. The third carbon source is the soil organic matter (SOM) already stored in complex recalcitrant substances. Soil microorganisms use those three pools as growth and energy source at different rates. Based on their importance for utilization by soil microorganisms, these three C sources can be ordered as follows: root exudates, litter, and SOM (Kuzyakov and Bol, 2006). Root exudates can be decomposed within a few hours (Jones et al., 2005). A considerable amount of the carbon assimilated by vascular plants through photosynthesis can be allocated belowground by root exudation and as plant litter. Wide range of labile carbon compounds are continuously released from the plant root system including mucilage, ectoenzymes, organic acids, sugars, phenolics and amino acids (Marschener, 1995). Once released to the soil, these compounds can serve as an easily available substrate for the methanogens and have a substantial effect on methane production in the soil (Joabsson et al., 1999). Although wetlands are rich in organic matter, a large fraction of the organic materiel at soil depths where methanogenesis takes place is old and recalcitrant (Hogg, 1992; Christensen et al., 1999). The substrates utilized by methanogens are limited to end products of the metabolic activities of other microbes. The effect of macrophyte on methane production is quite complex. The release of oxygen from roots to the rhizosphere inhibits methanogenesis (Watson et al., 1997) and also oxidizes methane (de Bont et al., 1978; King 1994). The oxygen released is not only used for methane oxidation but also in the oxidation of other reduced components (Fe (II), Mn (II), NH_4, H_2S). Methanogens are poor competitors with other heterotrophic microorganisms for limiting amounts of substrate, so that methane production normally commences after reservoirs of alternative electron acceptors are depleted (Actnitch and Rude, 1988; Capone and Kiene, 1988). As a consequence, activity of heterotrophic microorganisms other than methanogens may suppress methane production rates in substrate limiting environments such as salt and brackish systems with high abundance of sulfate (De Laune et al., 1983; Lovely et al., 1986; Bartlett et al., 1987; Middelburg et al., 1996) or Fe (II) rich freshwater systems (Lovely and Phillips, 1986; Burdige, 1993; Boon and Mitchell, 1995).

Influence of Plant on Methane Emission

Species Composition- Different species of plants differ in their capacity to influence methane production as well as emission. Plants influence composition and stimulate activity of microorganisms by producing labile carbon compounds through root exudation (De Nobili et al., 2001; Paterson, 2003). Plants differ in the amount and type of labile C released from living roots (Jones et al., 2004; Van der Krift et al., 2001) and in their competition with microbes for nutrients (Van Veen et al., 1989; Wang and Bakken, 1997). Thus plants play a selective role in enriching the microbial community in rhizospheric zone.

Biomass and Productivity- Plant biomass may affect CH_4 emissions, as organic material from plants may serve as substrate for methanogens. Strong correlations between sedge biomass and CH_4 emissions have been observed before (e.g. Klinger et al., 1994; Whiting and Chanton, 1992). Methane flux was found to linearly correlate with plant biomass (Whiting et al., 1991). Chanton et al. (1993), reported higher methane emission rate from *Typha domingensis* compared to *Carex jamaicense* due to the former's higher aboveground biomass and production. This suggests that quantitative differences in plant biomass and production rather than qualitative differences may control methane emission. Whiting and Chanton, (1993) reported a linear relationship between methane emission and net ecosystem production (NEP). With the help of modeling they found that 3% of NEP during peak production periods was emitted back to the atmosphere as methane.

Hyvoenen et al., (1998) studied the relationship between methane emission and standing biomass. They found that a correlation existed between methane emission and the daily maxima of Net Ecosystem Exchange (NEE) but not with the standing stock of *Equisetum fluviatile*. Their finding was in accordance with that of Mikkela et al., (1995) who found that correlation existed between the two as methane is mostly produced from recent biomass. They are of opinion that plant biomass does not appear to be the main controller of methane flux unless the transport capacity of the plant is saturated by methane.

Methane flux correlated with the number of green and brown *Eriophorum vaginatum* shoots, total shoot number, dry weight of above-ground biomass (green, brown and all

shoots) and below-ground biomass which was found to be the best predictor of CH_4 flux ($r^2 = 0.93$) (Greenup et al., 2000).

CH_4 emission was found to be sensitive to NEE and carbon turnover from an arctic wet tundra ecosystem in north-east Greenland, and the most plausible explanation was a combined effect of vegetative CH_4 transport and substrate quality coupled to vascular plant production. Total aboveground biomass correlated to mean seasonal CH_4 emission, but on separation into species, plant-mediated CH_4 transport was found to be highly species dependent (Joabssen and Christensen, 2001).

Stomata-The role of stomatal aperture in the context of methane emission has been debated. While Seiler et al., (1984) and Chanton et al., (1992b) found methane emission to be independent of stomatal aperture, Nouchi et al., (1990) observed a slight effect of stomata on methane emission. Knapp and Yavitt, (1995), Morissey et al., (1993) and White and Ganf, (2000), did find a positive correlation between methane emission and stomatal conductance in *Typha*. Nevertheless, stomatal aperture does play an important role in the transport of gases. Apart from stomata other vents are also found through which the gas is released. Kulshreshta et al., (2000) found micropores in *Scirpus* at the water– air interface which help in emitting methane. In rice plants, leaf blades form the main outlet through which 50% of methane is emitted before root elongation. Leaves are the major release sites at the early growth stage while nodes become more important later. Micropores were found in the on the leaf sheath and cracks at the junction of internodes forming outlets. Panicles generally contribute little to methane emission. Increasing water depth temporarily reduces methane emission while concentration gradients in rice plants readjust to unsubmerged emission sites. Methane emissions in rice plants cease only when the plants become totally submerged. Panicles form the main outlet when the vegetative parts are submerged (Nouchi and Mariko, 1993).

Environmental Factors

Environmental factors that affect the plant directly but methane emission indirectly are reviewed below.

Light- For several wetland plant species methane emission is higher under light than dark conditions. These diel patterns in methane emissions can be ascribed to changes

in sediment and air temperature (Schutz et al., 1989, Mikkela et al., 1995), plant transpiration rates (Chanton et al., 1997) and light intensity levels influencing the stomata (Chanton et al., 1993; Whiting and Chanton, 1996). Besides its effect on sediment and plant temperature, light may enhance methane emission rates by

(1) accomplishing a shift from diffusive-driven towards pressure-driven transport (Sebacher et al., 1985; Brix et al., 1992; Chanton et al., 1993; Chanton and Whiting 1996; Whiting and Chanton, 1996),

(2) increasing stomatal conductance (Knapp and Yavitt, 1992; Morrissey et al., 1993; Frye et al., 1994) and

(3) by increasing photosynthetically coupled methane production rates (Whiting et al., 1991; Whiting and Chanton, 1993; Minoda and Kimura, 1994; Chanton et al., 1995).

In a study by Brix et al., (1996), diel variation was observed in *Phragmites australis* with highest rate in the morning and the lowest rates during the night. Van der Nat et al., (1998) also found similar observations from *Phragmites* sp. and *Typha* sp. in constructed wetlands. They reported diel variations in methane emission, with highest emission rates at daytime and emission peaks following sunrise, for *Phragmites* sp. and *Scirpus* sp. The diel difference and magnitude of the emission peaks were much smaller for *Scirpus* than for *Phragmites*. The gradual increase of light intensities resulted in a gradual increment of methane emission from *Phragmites australis* (Van der Nat et al., 1998). *Cladium jamaicense*, which employs diffusive gas exchange, did not exhibit diurnal variations in methane emission rates although stomatal conductance varied diurnally (Chanton et al., 1993).

Temperature- It has an indirect influence on methane emission. A positive correlation has been found between diurnal and seasonal pattern of methane emission and soil temperature at 1 to 15 cm depth of soil (Schutz et al., 1989). Seiler et al., (1984) found a positive correlation at 0.5 cm soil depth whereas Cicerone et al., (1983) did not find any positive correlation at a depth of 23 cm. This suggests that processes leading to methane emission are mainly located in upper soil layer /surface and the roots of the plants are also concentrated in this zone. However Lindau, (1994), found no correlation between methane flux and soil temperature at 10 cm depth. Schutz et al., (1989) found that an increase of soil temperature from 20° to 25° C resulted in a doubling of the methane emission rates (Q_{10} approx 4).

Studies on methane emission from Wetlands in Asia

South-Asia is the major rice producer of the world. Paddy fields are one of largest sources in the global budget of CH_4 (Altor and Mitsch, 2000). Based on incubation experiments in a laboratory, Koyama (1963) first estimated that CH_4 production rate from world rice production was 190 Tg yr^{-1}. Since the 1980s, there have been numerous field measurements of CH_4 fluxes in various rice paddies over the world (e.g. Cicerone and Shetter, 1981; Holzapfel-Pschorn et al., 1986; Schütz et al., 1989; Sass et al., 2002; Yagi and Minami, 1990), leading to revised estimates of the global CH_4 emission from rice paddies to 60 Tg yr^{-1}, but with uncertainty ranging from 20 to 100 Tg yr^{-1} (IPCC, 1995). The review below is limited to studies made on methane from different wetland ecosystem apart from paddy fields.

Vembanad Lake contributes significant amount of methane to the atmosphere. Average emissions varied spatially within the lake. Methane emissions were 193.2±24.5 mg m^{-2} h^{-1} at Kumaragam (fresh water) as compared to 9.3±9.6 mg m^{-2} h^{-1} at Pullot (brackish water) site. Seasonal variation was significant between pre- and post-monsoons. Soil temperature, time of the day, salinity sediment organic carbon, all control the rate of methane emissions from the Vembanad Lake. Mangroves are generally considered a minor source of methane but Purvaja et al., (2004) showed that the freshwater influenced polluted Pichavaram mangroves emit methane close to the Northern wetlands (a major emitter).

In profundal sediment samples collected from Lake Kinneret (Israel), methane production rates were higher at 30°C than at the in situ temperature of 15°C and were higher in the top 5 cm layer than below. 30 to 50% of total CH_4 production was from reduction of CO_2 (Nusselein et al., 2001).

In Lake Biwa (Japan), methanogenic activity was much higher in the littoral sediments compared to the profundal sediment (Murase and Sugimoto, 2002). The bottom water of the littoral sediment was also capable of methane oxidation. Methane oxidation was also observed in the column of hypolimnetic water but very low oxidation found in the epilimnion and thermocline due to inhibitory effect of light (Murase et al., 2005). They found that more than 90% of methane was oxidized while

diffusing through the littoral sediment and water column. Hydrogenotrophic methanogenesis pathway dominated methane production (Murase and Sugimoto 2001).

Flux rates of carbon dioxide (CO_2) and methane (CH_4) were studied at various peat water table depths in a mixed-type peat swamp forest floor in Central Kalimantan, Indonesia. Methane emissions from the peat surface remained small and were detected only in water-saturated peat. By applying long-term peat water table data, annual gas emissions from the peat swamp forest floor were estimated to be 3493± 316 $gCO_2\ m^{-2}$ and less than 1.36±0.57 $gCH_4\ m^{-2}$ (Juhaiinen et al., 2005).

Diurnal and seasonal variations in carbon dioxide and methane fluxes between Sundarban biosphere and atmosphere were measured using micrometeorological method during 1998–2000. The mixing ratios of methane were found to vary between 1.42 and 2.07 ppmv; and that of carbon dioxide, between 324.3 and 528.7 ppmv during the study period. The overall annual estimate of carbon dioxide and methane fluxes from this ecosystem to atmosphere were estimated to be 694 Tg yr^{-1} and 184 Gg yr^{-1}; respectively (Mukhopadhyay et al., 2001; 2002).

Wang and Han, (2005) compared diurnal methane fluxes between a sandy and organic site. The measurements were taken in summer and winter. In summer, environmental variables like water table, temperature mediated by the solar radiation was found to correlate with the diurnal fluxes from the sandy site. Plant photosynthesis greatly affected the mechanisms of CH_4 production, oxidation and transport, resulting in a diurnal variation of CH_4 emission with a peak in the late afternoon and the lowest value immediately prior to sunrise of the next day. In the organic site, vascular plants were found to control the diurnal variation in methane flux.

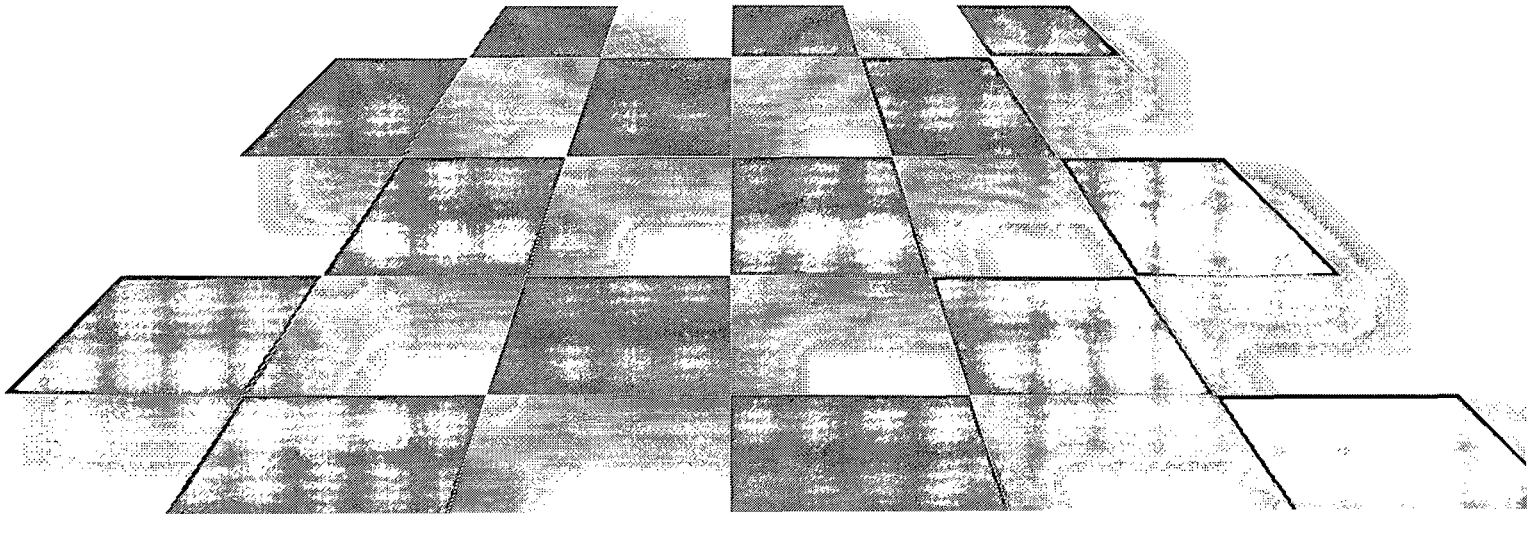
Ding et al., (2003) studied the summertime variation pattern of CH_4 oxidation in the rhizosphere. CH_4 oxidation was evaluated in a freshwater marsh vegetated with *Carex lasiocarpa* and *Carex meyeriana* in the Sanjiang plain of northeast China at the three growing stages.

In 2004, Ding et al., studied the diel variation in methane emissions from the stands of *Carex lasiocarpa* and *Deyeuxia angustifolia* in a cool temperate marsh in Sanjiang plain.

Hirota et al., (2004) measured the methane emissions from different vegetation zones in a Qinghai-Tibetan Plateau wetland during the plant growth season (early July to mid-September). They employed static chamber for measurements of methane flux from four vegetation zone along a gradient of water depth. They observed that the smallest methane flux (seasonal mean 33.1 mg CH_4 m^{-2} day^{-1}) was in the *Potamogeton* dominated zone, which occupied about 74% of the total area of the wetland and the greatest methane flux (seasonal mean 214 mg CH_4 m^{-2} day^{-1}) in the *Hippuris*-dominated zone, in the second-deepest water area.

Zhang et al., (2005) studied CH_4, CO_2 and N_2O fluxes in cold season (winter and thaw) from freshwater marshes in Sanjiang Mire wetland (47°35'N, 133°31'E, Northeast China), using the static chamber method. The contributions of winter CH_4 fluxes were about 5.5% and 3% in the *Carex lasiocarpa* and *Deyeuxia angustifolia*, respectively. Marshes are an important potential N_2O sink in winter season in northeast China. During the thaw, the CH_4 and CO_2 emissions rapidly increased, 4.5 to 6 times of winter emissions.

MATERIAL AND METHODS



Chapter III
MATERIAL AND METHODS

Sampling Sites

Enroute from Dalhousie (13km) to Chamba in Himachal Pradesh lies a beautiful meadow with **Lake Khajjiar** amidst dense forest of *Cedrus deodara*. The lake is located at 32°33' N Latitude and 76° 35' E Longitude (Fig.2A). The elevation of the lake is 1950 m a.s.l and the lake is covered with snow for nearly six months. The mean monthly temperature ranges from 2°C (minimum -8.6°C) to 25.5°C (maximum 35.5°C). The lake is situated in middle of a trough shaped glade and receives the major runoff from the surrounding Deodar forest. The marshy area around the lake is lined with vegetation, which in turn is surrounded by a meadow. Beyond the meadow stands the mixed Deodar forest. The peat accumulated in this lake is basically due to the deposition of undecomposed vegetation matter comprising mainly of *Acorus* sp, *Cyperus* sp, *Phragmites* sp *Scirpus* sp, etc. The rate of methane production from soil at this lake was studied for two consecutive years and the influence of plants on methane emission was studied in the second year.

Lake Bhalswa, in North-East of Delhi, is a freshwater wetland containing mineral sediments. It is lined with *Scirpus littoralis* in its periphery. The lake is located at 28°74' N Latitude and 77° 17' E Longitude (Fig.2A). Monthly mean temperatures range from 14.3°C in January (minimum 3°C) to 34.5°C in June (maximum 47°C). Mineral soil was transported to the School of Environmental Sciences, JNU where the experiments were setup in a microcosm.

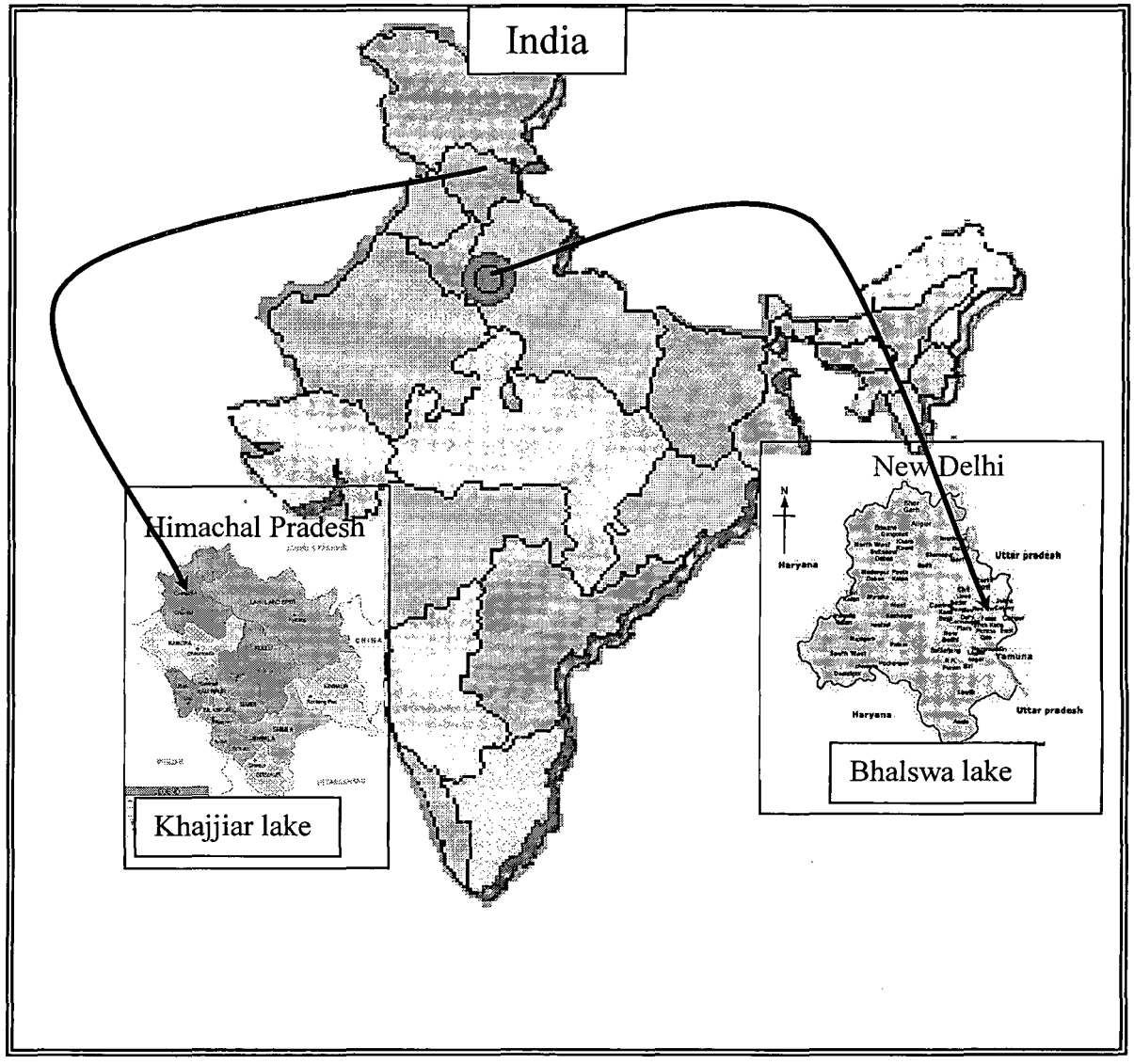


Fig.2A. Location of Sampling Sites in India: Lake Khajjiar in Himachal Pradesh and Lake Bhalswa in Delhi.

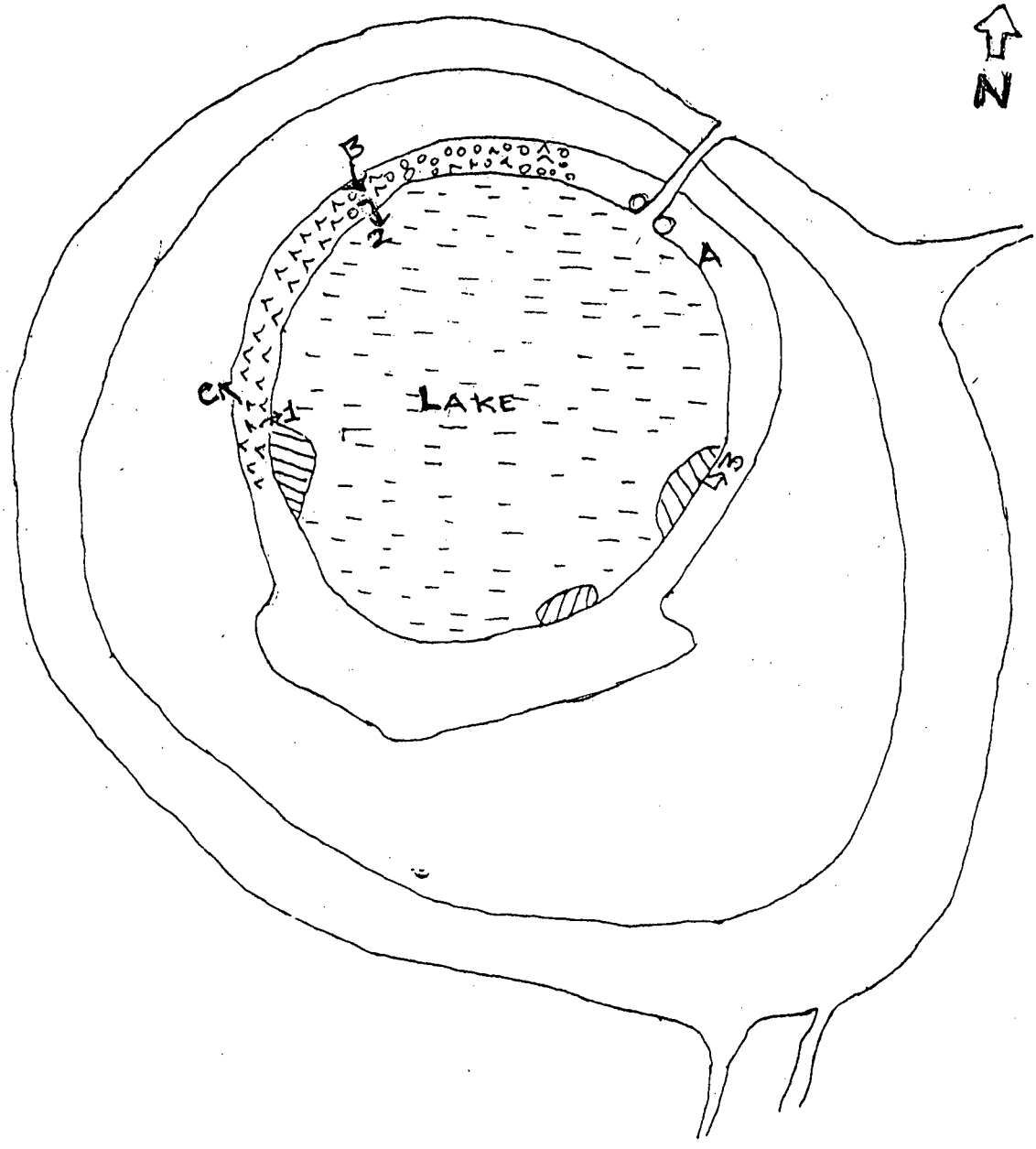


Fig.2B Sites at Lake Khajjiar

Sites A, B and C sampled at June, 2004.
Sites 1, 2 and 3 sampled at August, 2005.

Index of vegetation present at sites: *Phragmites* sp. *Acorus* sp

Cyperus sp.

Collection of Soil

Sampling of peat at Lake Khajjiar in June, 2004

Peat soils were collected from three sites: south-eastern side on the left of boardwalk when facing the lake (henceforth site-A), north-western side (henceforth site-B) and the western side (henceforth site-C) around the periphery (within 5 m from the water of the Lake) of Lake Khajjiar in June 2004. Sites A and B were covered with mosses whereas the site C was amidst dense vegetation of *Acorus* sp. The sites were about 25 m apart and the sites A and C were diagonally opposite to each other across the lake (Fig.2B). Water table was about 2 cm below the surface and water oozed out as one walked on it. A 10×10 ×10 cm corer was pressed in the soil. The peat compressed in the corer came out in a compact form when the corer was pulled out. Blocks of peat at a depth of 0-10, 10-15cm and 15-20 cm were dug out with a long trowel. After collection the soil was packed in polythene packets and transported to the lab in Max Planck Institute, Marburg. The experiments with soil collected in, 2004 were conducted at Max Planck Institute of Terrestrial Microbiology, Marburg.

Sampling of peat at Lake Khajjiar in August, 2005

Peat soils were collected from three different sites: in the western side of the Lake, (henceforth Site-1), the north-western side. (henceforth Site-2); the eastern side (henceforth Site-3) (Fig.2B) around the periphery (within 2-3 m from the water of the Lake) of Lake Khajjiar. During the two visits to Lake Khajjiar, the area denoted as Site-C (on the western side) and Site B in the first sampling in June 2004 is closer to the area denoted as Site-1 and Site-2 respectively, in the second visit in August, 2005. Blocks of peat at a depth of 0-5, 5-15cm and 15-25 cm were dug out with a long trowel. After collection the soil was packed in polythene packets and transported to the lab.

Sampling of mineral soil at Lake Bhalswa in January, 2006

Mineral soil was dug from periphery of Lake Bhalswa and transported to the School of Environmental Sciences, JNU. Experiments were set up in tanks of 54 x 54 x 57.5 cm with Lake Bhalswa soils laid at 13-15cm depth and used for growing *Scirpus* plants.

Procedure of collection of methane samples from field

Methane samples were collected from the field using the chamber method.

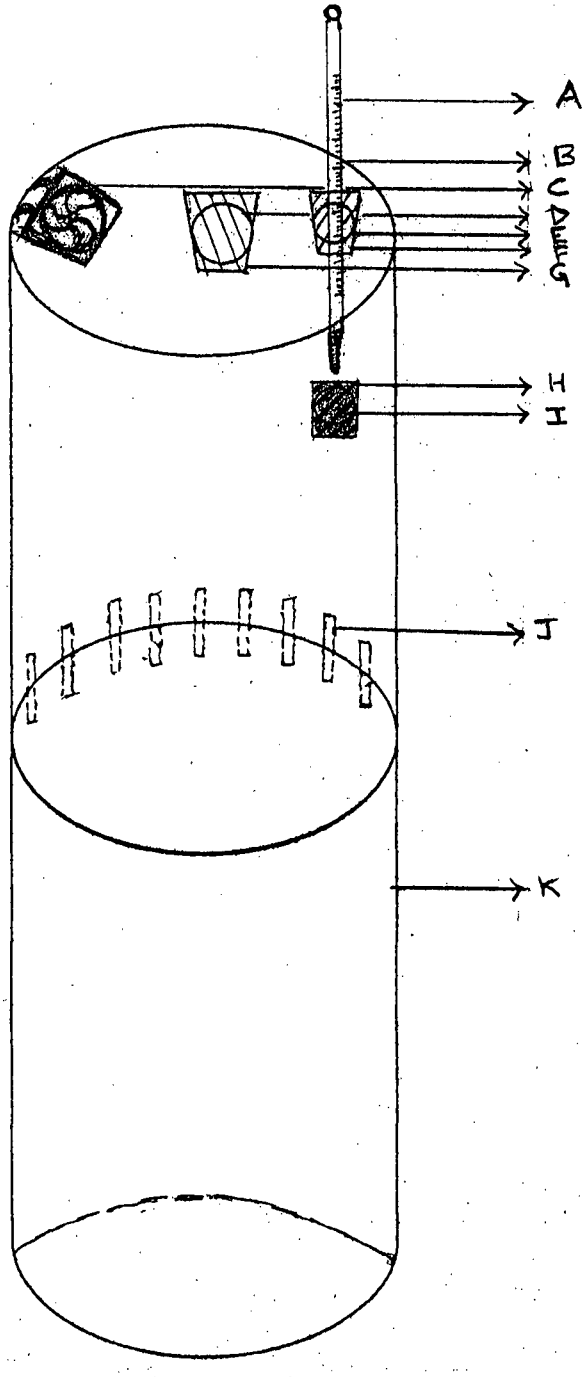


Fig.3 Diagram of a chamber: A-Thermometer, B-Upper module of the chamber, C-Fan, D-Central vent, E-Vent for thermometer, F-Rubber cork with fixed thermometer, G-Rubber cork, H-Silicon rubber strip, I-Vent for syringe, J-Vertical strips shown on one side only, K-Lower module.

Chamber Design

The chambers were made of cylindrical Perspex glass of the dimension 61cm in length and 31.6cm in diameter (Fig. 3). Each chamber had two distinct modules (L and K) that could be fitted one on top of another. The upper module (L) had a transparent sheet of Perspex glass sealed on the top of the cylinder. A fan (C) was fixed on the top sheet (inner side) for air circulation. Two vents (D and E) of different sizes were drilled in the top sheet. The smaller hole (E) was used for inserting the thermometer embedded in a rubber cork and the larger hole in the center (D- sealed with a rubber cork G) was used as a vent for flushing of air. A small hole (I) drilled on the side was sealed with a square silicon rubber septum (H) and served as a port for withdrawing sample. The lower module consisted of the Perspex cylinder attached with vertical strips (J-15 cm) of Perspex sheet in the inner side that gave support during the attachment of the two modules. Both the modules were carefully joined with selotape and were sealed freshly everyday the chamber was used. The lower module was attached to the upper one when the plants were too tall to fit within the upper module. With the growth of the plants another cylinder without a top sheet was placed below the cylinder that contained the top sheet. The sealing of the top chamber was checked regularly.

PVC pipes of 3.7cm internal diameter, 60 cm length with fabricated polypropylene cap containing circular sheet of silicon rubber (for collecting gas sample with syringe) attached (silicon gel used) to the outer ring of the polypropylene cap were used to collect gas from bare soil at Lake Khajjiar.

The peat soil as loose enough to allow the chambers to penetrate the soil, the water that came out on pressing the chambers in the peat acted as a sealant for the trapped gas in the chamber. At Site-2, where the chamber could not cut through the peaty soil, soil from the side was taken and used for plugging the chamber in all side. To minimize the disturbance caused by walking while setting the experiments and sampling, planks of wood were placed and used as a platform for walking. Static box chamber method was used (described above) to enclose the plants. The chambers and pipes were placed in the soil at least half an hour before sampling without the rubber corks to equilibrize the internal atmosphere of the chamber (containing higher

methane concentration due to soil disturbance) with the external atmosphere. Samples were collected every 15 min for one hour from Site 1and 2. In Site-3, the samples were collected every half an hour for two hour. A 60 ml plastic syringe with three-way valve was used for sampling gas from the chamber. The gas was injected in the vial unless a backpressure was felt. Stoppered and crimped glass vials of 60 ml were used for storing gas. These glass vials were evacuated by a vacuum pump the night before sampling. After sampling the vials were stored in cold during transportation and in less than 10°C before analysis.

In the experiments conducted in the cement tanks (with different types of soil), square aluminium frames were inserted in the soil before planting the saplings of *Scirpus* plants to minimize soil disturbance during methane sampling. As mentioned above the same procedure was followed for collecting and stroring the gas. The samples were collected every half an hour for two hours. The vials were kept in a thermocol box containing ice for immediate storage. The vials were then stored at 4°C until analysis within one to three days.

Flux Calculation

Standard for methane gas was prepared by mixing known quantity of pure methane (Sigma Gases, Delhi) with a known quantity of pure nitrogen (Sigma Gases, Delhi). The concentration of methane from unknown samples is determined by correlating the peak area with the standard graph obtained for known concentrations of standard methane. The methane flux was calculated from the temporal increase of the methane mixing ratios inside the box using the equation:

$$\text{Methane flux (F)}(\text{mg m}^{-2}\,\text{hr}^{-1}) = \frac{BV_{STP} \times C_{CH4} \times 16 \times 1000 \times 60}{10^6 \times 22400 \times A \times t}$$

where, BV_{STP}(Box Air Volume in cc at STP)$= \frac{BV \times B.P. \times 273}{(273+T) \times 760}$

Box air volume, $BV = [\pi r^2 (H-h)$ -Biomass volume inside the box];

r=Radius of the Chamber

H = Chamber Height;

h = water level above channel;

B.P. = barometric pressure (mm Hg);

T = box air temperature at the time of sampling in °C;

C_{CH4} = change in CH_4 concentration in ppmv from 0 minute to the t minute sampling ;

and A = area covered by the chamber in m^2.

This formula was adapted from the work of Parashar et al., (1996). The temperature inside the chamber was noted but due to non-availability of an instrument, pressure was not recorded. As pressure varies very little during the study period, atmospheric pressure was not considered in this study.

In incubation experiments, slopes of linear regression of total gas production versus time were used to calculate the production rates of each gas, after correcting for pressure, temperature and solubility. At the end of incubation the slurry was dried at 60° C and the rate of production was normalized to per gram of dry soil. For the calculation of slope linear regression of CO_2, the quantity of dissolved form of bicarbonate was also taken into consideration where the rate of carbon dioxide was determined.

Procedure of methane sampling under laboratory condition

The sample bottles were shaken manually to allow equilibration between the liquid and the gas phase and then gas samples were drawn from the bottles. Pressure lock syringes ranging from 0.25µl to 1 ml to was used to withdraw the sample.

Analysis

Analysis of gas samples

At Max Planck Institute (MPI), Marburg, methane, carbon dioxide and hydrogen were measured in Gas Chromatography SRI-9300 (SRI Instruments, Torrance, USA) equipped with Flame Ionization Detector. The gases were separated in a Porapak QS Column of 80/100-mesh size, with a temperature of 80°C. A methaniser (Chrompack, Middelburg, Netherland) with the column temp of 350°C was attached to the Gas

Chromatograph to measure CO_2. Hydrogen was measured in a Gas Chromatograph GC-8A with Thermal Conductivity Detector. The carrier gas was nitrogen with a flow rate of 20 ml s^{-1}.

At JNU, the samples were analyzed for methane in the Flame ionization detector of Gas Chromatograph (Perkin-Elmer Auto System XL). Nitrogen flowing at a rate 30 cm/sec in the porapak Q column acted as a carrier gas. The oven temperature was set at 45 °C, injection temperature was set at 80°C and the gas was detected at a temperature of 150°C.

Analysis of soil samples

The pH was measured using a digital pH meter.

Organic Matter Content

Organic matter content of the soils was analysed by loss on ignition method (500°C) (Heiri et al, 2001).

Fatty acid analysis

At MPI, the aqueous phase of the soil was extracted after centrifugation at 4°C for forty-five min. The pore water collected as supernatant, was filtered through a 0.2μm membrane filter, and then stored at –20°C until further analysis of short chain fatty acids (acetate, propionate, butyarate). Fatty acids were measured in High Pressure Liquid Chromatography with a Refractive Index detector (Schambeck SFD GmbH) and Ultraviolet detector of 205nm wavelength. (Uvis 200, Linear Insrtuments, Reno USA). Sulfuric acid (0.1mM) was used as an eluent. The column used was of stainless steel packed with CARBOSep CAR-H procured from Schambeck SFD GmbH, Germany. The chromatograms were analysed using a software program Peak Simple-Ver.4.9.

Estimation of Total Nitrogen

0.3 g of dried, grinded, and sieved soil was put in the digestion tubes, 2 ml digestion mixture was added and digested on the thermal block for three hour at 360°C. the temperature was raised slowly and the digestion was carried out until the colour of the mixture turned light apple green. After cooling the tubes, 50 ml water was added to the tubes. The mixture was decanted or filtered and the volume was made up to 100ml with distilled water. 0.1 ml of this mixture was used to determine total nitrogen colorimetrically. 5ml of N1 reagent (1000 ml of distilled water containing 34g sodium

salicylate, 25 g sodium citrate, 25g sodium tartarate and 0.12 sodium nitroprusside) is added to 0.1 ml of the digest in a test tube. The reagents in the tubes were mixed well and were allowed to stand for 15 min. 5 ml of N2 reagent (1000 ml of distilled water containing 30g sodium hydroxide and sodium hypochlorite) was added to the digest. The reagents in the tubes were mixed well and were allowed to stand for 1 hour until the colour development was complete. The absorbance was taken against 655nm in a spectrophotometer. A blank was used every time digestion was done and it only contained digestion mixture and no soil. The rest of the procedure was same as the sample. The blank value was subtracted from sample value before processing the data. The concentration of the total nitrogen in the sample was determined from slope value of a graph where the absorbance is plotted against corresponding known concentration of nitrogen (in the form of ammonium sulfate) (Anderson and Ingram, 1989).

Estimation of Total Phosphorus

5ml clear digest from the above digested sample was used to determine the total phosphorus present in the sample. 20ml distilled water was added to 5 ml digest in a 50ml volumetric flask, 10 ml of ascorbic acid reducing reagent is added. The volume was made up to 50ml by adding distilled water. The flask was stoppered, shaken well and left undisturbed for 1 hour for the full development of the colour. The absorbance of the samples was taken at 880nm in a spectrophotometer. A blank was used every time digestion was done and it only contained digestion mixture and no soil. The rest of the procedure was same as the sample. The blank value was subtracted from sample value before processing the data.

The concentration of the total phosphorus in the sample was taken from slope value of a graph where the absorbance is plotted against corresponding known concentration of phosphate (in the form of potassium phosphate) (Anderson and Ingram, 1989).

Estimation of Nitrate (Inorganic Form) 50 g soil was put in 500ml wide mouthed beaker. 0.5 g calcium sulfate and then 250 ml double distilled water added. The bottle with its content was shaken for 10 min; the suspension was allowed to settle for a few min and filtered. 25 ml of this filtrate is added to a beaker containing 0.05g of calcium carbonate and the sample was dried completely in a very low temperature (60°C) mostly when kept over night. The beaker was cooled and 2ml of phenoldisulfonic acid (prepared by dissolving 25g of pure white phenol in 225 ml concentrated sulfuric acid) added. The beaker was rotated and allowed to stand for 10 min. 20 ml double distilled

water was added while stirring with a glass road. Ammonium hydroxide was added slowly from a biurette until yellow coloured developed. The solution was then transferred to a 100ml volumetric flask and the volume made up to 100ml by adding double distilled water. The intensity of the yellow colour was measured at 420 nm against reagent blank (Bremner, 1965).

The concentration of the nitrate in the sample was taken from slope value of a graph where the absorbance was plotted against corresponding known concentration of nitrogen (in the form of potassium nitrate).

Estimation of Nitrite (Inorganic Form)

The filtrate obtained for nitrate analysis was used for colorimetric analysis of nitrite. 2ml of the filtrate was pipetted into a 50ml volumetric flask, double distilled water was added to make the volume to about 45ml. 1ml diazotising agent (containing 0.5g sulphanilamide in 100ml 2.4 N hydrochloride solution) was added. After 5 min a coupling reagent (containing 0.3g N-(1-napthyl) – ethylenediamine in 100ml 0.12N hydrochloric acid) was added. The solution was mixed well and after 20 minutes the intensity of the colour measured against 520nm (Bremner, 1965).

Estimation of Microbial Biomass Carbon Fumigation Extraction method was used to estimate the microbial biomass carbon. 25 g of fresh soil was incubated with pure chloroform in a dessicator for 24 hours in dark condition. Another, 25 g soil was shaken with 100 ml 0.5M Potassium sulfate (K_2SO_4) solution for half an hour. The solution was filtered through an ashless filter. 8 ml of the filtrate was digested with 0.007g mercury oxide, 2 ml of 66.67 mM potassium dichromate ($K_2Cr_2O_7$), 10 ml of 18M sulphuric acid (H_2SO_4), 5 ml of 14.7 M phosphoric acid (H_3PO_4). The solution was boiled under refluxed conditions for 30 minutes. The excess dichromate remaining after digestion was titrated against 33.3mM ferrous ammonium sulfate using ferroin as an indicator. The fumigated soil was aired properly and then the same process was followed for the unfumigated soil (Vance et al., 1987). The carbon content of the unfumigated soil represented the readily mineralizable carbon. The difference in the carbon content of the fumigated and unfumigated soil represented the microbial biomass carbon.

Estimation of Total Organic Carbon

0.5g organic soil or 1g sandy soil was allowed to stand overnight with potassium dichromate (1N). The unreacted potassium dichromate was titrated against freshly

prepared ferrous ammonium sulfate (0.4 N) in presence of phosphoric acid (10 ml), sulphuric acid and diphenylamine (indicator) (Trivedy and Goel, 1984).

Estimation of Soil Texture

The soil texture was analysed using Bouyoucos hydrometer method (Singh, 1989).

Soil Temperature

The temperature of the soil 5 cm below the surface was noted every half an hour during methane sampling from vegetated and bare soil with a thermometer.

Molecular analysis of methanogens

T-RFLP technique was used for the detection of methanogens in the pre-incubated soil of three layers at Site A and B; soil incubated with and without methyl fluoride from the 0-10cm layer at Site-A and soil incubated with and without methyl fluoride from the three different depths (0-10, 10-15 and 15-20cm) at Site B at Lake Khajjiar.

DNA Extraction and PCR amplification

Soil samples (from Lake Khajjiar collected in June 2004) before and after the incubation of different temperatures were collected and stored at –20°C until DNA extraction. The stored soil was thawed, homogenized in a clean mortar and pestle. 300 mg of this soil was used to extract DNA by mechanical (bead beating) and chemical (detergent) cell lysis with the Soil DNA Isolation kit. The protocol was followed as suggested by the manufacturer (Fast DNA SPIN Kit for soil; B10101 Systems; QBiogen)) but with minor modifications. Guanidine thiocyanate was used in the intermediate steps to remove the interference of the humic acid. The yield of the DNA was checked in 1% agarose gel stained with ethidium bromide.

One microliter of the extracted DNA was used for the amplification of the archaeal small subunit (SSU) rRNA encoding gene (rDNA) in an Eppendorf Mastercycler Gradient –5331,Ver.No.2.12.31. The primers used to amplify specifically an approximately 800 bp long region of methanogen 16S rRNA gene were 109f and 915r FAM (6-carboxyfluoroscien) labelled (MWG). The PCR mixture contained 10X Buffer, Bovine Serum Albumin and dNTPs-5µl each, $MgCl_2$-3µl, Primers-0, 5µl(109f and 912rt-Grosskopf et.al 1998;Leuders and Friedrich,2002), Taq Polymerase (Promega)-0.25µl and the volume was made up to 50µl by PCR water (Sigma). BSA

($4\mu g\mu l^{-1}$) was used to prevent PCR inhibition. The PCR was programmed for 3 min hot start at 94°C followed by denaturation for 30s at 94°C; annealing at 54°C for 45s and then extension at 72°C for 1min and 30s.This cycle was performed for 30 times before the final extension again at 72°C for 7min. Amplified DNA was verified by electrophoresis in 1.0% agarose in 1X TAE buffer and stained with ethidium bromide. The amplified archaeal DNA product (amplicon) was purified by using binding and washing buffer (Buffer PE) and later collected by elution buffer (10mM Tris-Cl, pH 8.5) according to the manufacturers instruction in MiniElute PCR Purification Kit (Qiagen). The purified DNA product was quantified photometrically for dsDNA and accordingly 50 nanogram of the purified PCR product was digested with Taq-1 restriction enzyme (Promega) at 65°C for three hours.

T-RFLP

The digested amplicon (2.5μliter) was mixed with formamide (loading dye) and an internal lane standard consisting of 17 different 6-carboxy-X-rhodamine (ROX) – labelled fragments ranging in length from 29 to 928 nucleotides. This mixture was denatured for 3 min at 93°C before loading on to polyacrylamide gel. The electrophoresis was performed for 6 hrs on an automated ABI DNA sequencer (model 373; PE Applied Biosystems) under the following conditions: 2,500 V, 40Ma, and 27W. The laser scanning system of the aforesaid instrument detected the fluorescently labeled 5'-terminal fragment. The data was analysed using GeneScan analysis software (version 2.1).

Analysis of T-RFLP Profiles

The relative fraction of the peak height of each fragment size was calculated. An average was done in case of multiple analyses for the same set of samples.

Estimation of Biomass of plants

At the end of sampling at Lake Khajjiar in August, 2005 few plants of the three species were uprooted and the above and belowground biomass was estimated by drying in the oven at 65°C in laboratory. The number of shoots and their leaf length present inside the chamber was always measured on the days of sampling. Later the total biomass (g dry weight) and volume of the plants enclosed in the chambers were

calculated from the linear regression equation derived from the correlation between the volume and dry weight from the samples of each individual plant species (that were carried back to the laboratory). The regression equation (coeffecient of correlation= 0.94) used for calculating the biomass (g dry wt.) of *Acorus* plant was y=0.0215x-5.682 where y is the leaf length (cm) of sample plants and x is the biomass (g dry wt.) of the leaf or shoot. Similarly the linear regression equation (coefficient of correlation = 0.98) used for calculation of volume (cm^3) of plants present in the chamber was y=0.2119x-59.45 where y is volume (cm^3) and x is leaf length (cm).

A non-destructive linear method was used for the calculation of the biomass of *Scirpus* plants in the experiment conducted in the garden of School of Environmental Sciences (Vander Nat et al., 1998b). The number of green shoots and their leaf length present inside the chamber was measured on the days of sampling. Later the total biomass (g dry weight) and volume (cm^3) of the plants enclosed in the chambers were calculated. The regression equation (coeffecient of correlation is 0.97) used for calculating the biomass (g dry wt.) was y=0.0214x-0.8652 where y is the leaf length (cm) of sample plants and x is the biomass (g dry wt.) of the leaf or shoot of plants. The green leaves were only included in the calculation of biomass. The volume was also calculated by non-destructive method. The diameter at the lower portion of the plant shoots was noted with a screw gauge. The plants were categorized into six groups according to the diameter and length. The biomass volume (cm^3) was calculated by using the following formula:

$$\text{Biomass Volume} = 1/3 \times 3.14 \times r^2 \times l$$

where r is the radius of the plant (cm) shoot in its lower end and l is the length of the shoot (cm).

Study of methane emission from Lake Khajjiar

Methane samples were collected from bare soil and dominant vegetations present in the inner zone (2-3m from the water of the lake) from Sites-1, 2 and 3 around the lake in August, 2005.

Experimental Layout

The experimental design was concise and framed to fulfill the following objectives:-

1) To measure the rate of emission methane from the dominant vegetation and from bare soil
2) To see the spatial variability in emission

Methane was sampled from the in-situ dominant vegetation present at Site-1 - *Acorus* and *Phragmites* sp., Site-2 - *Acorus* sp. and *Cyperus* sp. and Site-3 - *Phragmites* sp. The samples were collected in triplicate for all plants except from that of *Phragmites* sp. in the site-1. Bare soil was also sampled in triplicate from the three sites. The water table lied around 2 cm below the peat surface in Site-1, 5-8 cm below the peat surface in Site-2, and just below the surface in Site-3.

In a clockwise manner, Site-1 (*Acorus* and *Phragmites* sp. and bare soil) was sampled in the first hour from 8-9 a.m, Site-2 (*Acorus* sp. and *Cyperus* sp. and bare soil) followed from 11-12 a.m. and then Site-3 (*Phragmites* sp. and bare soil) was sampled at 2-4 p.m. The sampling was conducted for three consecutive days at each site at same points.

Methane Emission as influenced by different soil types planted with *Scirpus* sp.

Based on an earlier study with different wetland plants, I found *Scirpus* species to be very convenient to handle and grow for experimental purposes. *Scirpus littoralis* is a perennial plant, found in wet places, often growing in standing water, tolerating depths of 2 m (Cook, 1996). *Scirpus* plants were grown in the tanks (54cm × 54 cm × 57.5) in the garden behind School of Environmental Sciences.

Three types of soil were used to grow the plants-

a) soil mixed with animal manure (referred as garden soil),
b) soil mixed with sand (referred as Sandy soil) and
c) soil collected from Bhalsawa Lake (referred as Bhalsawa soil).

The soil in each tank was 13-15 cm deep. Young *Scripus* plants of the same size (18-20 cm) were collected from wetlands in and around Delhi, sown in the tanks in March 2006. Depending on the availability of tanks, three replicates with each soil type was

used for growing the plant and one tank was left unvegetated to serve as control. The water in the tank was maintained around 8-10 cm above the water surface. Sampling for studies on methane was done in May, June, July and October 2006, except from the plants sown in Garden soil where sampling began from June.

An experiment was carried out to study the effect of water level on methane emission from *Scirpus* plants. Six tanks planted with Scripus were used. In the first two the water level was 0-5cm below soil surface, in the second two, the water level was maintained at 15 cm and the in the last two tanks the water level was maintained at 30 cm above the soil surface. The flux was studied for ten days and the rate of emission was measured at regular intervals.

Methane oxidation under laboratory condition

The oxidation of methane from different part of the shoot, roots and rhizospheric soil was assessed (Fig.4).

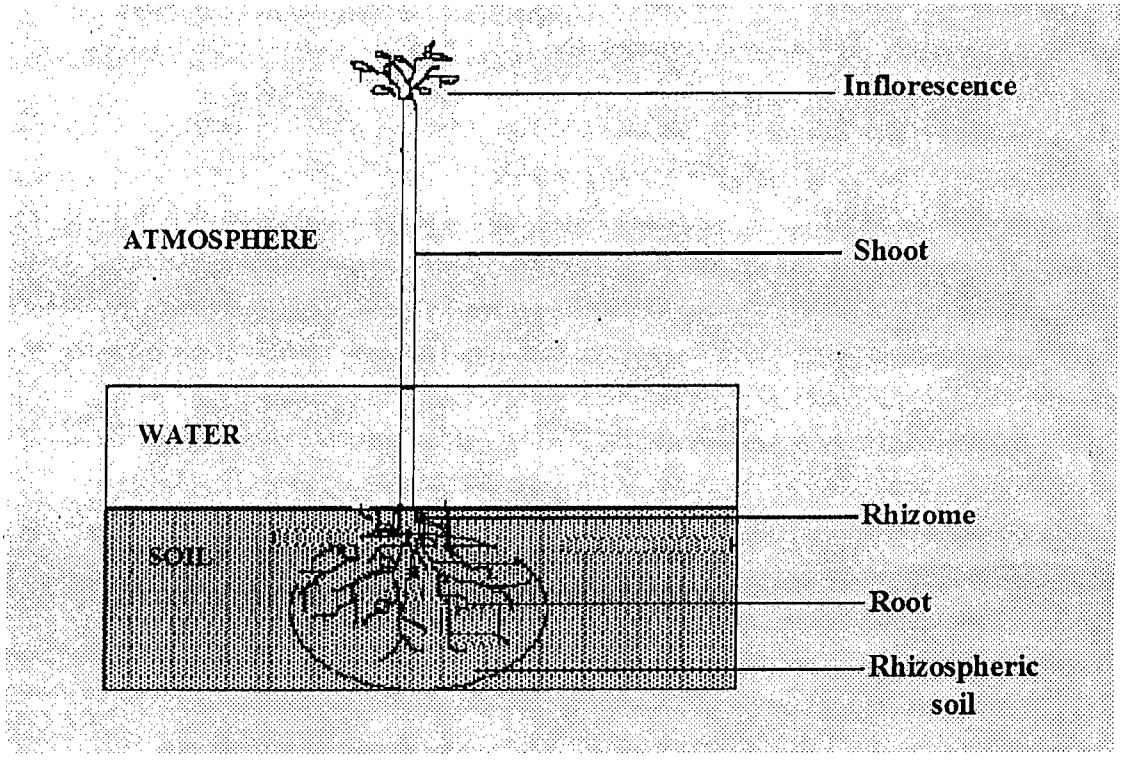


Fig. 4 Parts of *Scirpus* plants

The stem was cut in to sections of 6 cm till 36 cm plant length and enclosed in the glass vial. The glass vials were stoppered and sealed with aluminium crimp. 0.5 ml pure methane was added. The uptake was monitored at regular interval in a gas chromatograph and expressed for plant dry weight. The roots and rhizospheric soil was incubated similarly with methane and the uptake was calculated from the slope of linear regression.

Study of the response of methanogens in presence of different alternate electron acceptors and substrates

An experiment was carried out to study the response of the methanogens in the presence of different alternate electron acceptors at different soil depths (0-5, 5-15 and 15-25cm) at Lake Khajjiar and also in Bhalswa soil. Soil slurries were prepared by mixing the soils with solutions (in a ratio of 1:3) of sodium nitrate (10mM), sodium sulfate (10mM), sodium molybdate (10mM). Another set of experiment was conducted to observe the response of additional substrates like sodium acetate (500μM) and H_2/CO_2 on methanogens. The bottles were closed with rubber cap and sealed with aluminium crimp. Flushing with pure nitrogen gas deoxygenated the soil slurry. For the incubation with H_2/CO_2 (1:4 ratio), the soil slurry was made with double distilled water, flushed with pure nitrogen gas and then 1 ml of H_2/CO_2 was added. The bottles were incubated at 27°C. Triplicates were used for each set of electron acceptors. 1ml of gas was injected at regular interval until the slope was found to stagger. Linear regression was used to calculate the rate of potential methane production after correction for molar volume and was expressed in mg per g dry wt of soil per day.

Study of methane production potential at different temperature under laboratory condition

Soils from different depths (0-10, 10-15 and 15-20cm) of Site A and B at Lake Khajjiar were incubated at six different temperatures (4°C, 11°C, 15°C, 25°C, 37°C and 45°C) to investigate the influence of temperature on methane production. Slurries were prepared by mixing soil with autoclaved distilled water (under nitrogen atmosphere) in 1:3 (weight by volume) ratio. The slurry (20-25ml) was distributed in serum bottles (150ml), closed with rubber septa. The bottles were then flushed with

nitrogen and incubated in the following six temperatures 4, 11,15, 25, 37 and 45°C. Bhalswa, Sandy and Garden soil were incubated at four different temperatures (10, 27, 37 and 48°C) to study the variation in methane production under different temperature gradients. Triplicates were used. The head space gas namely methane, carbon dioxide was measured weekly for a month for site A and for a month and a half for site B. Steady state concentration of the hydrogen gas was measured at the end of the experiment

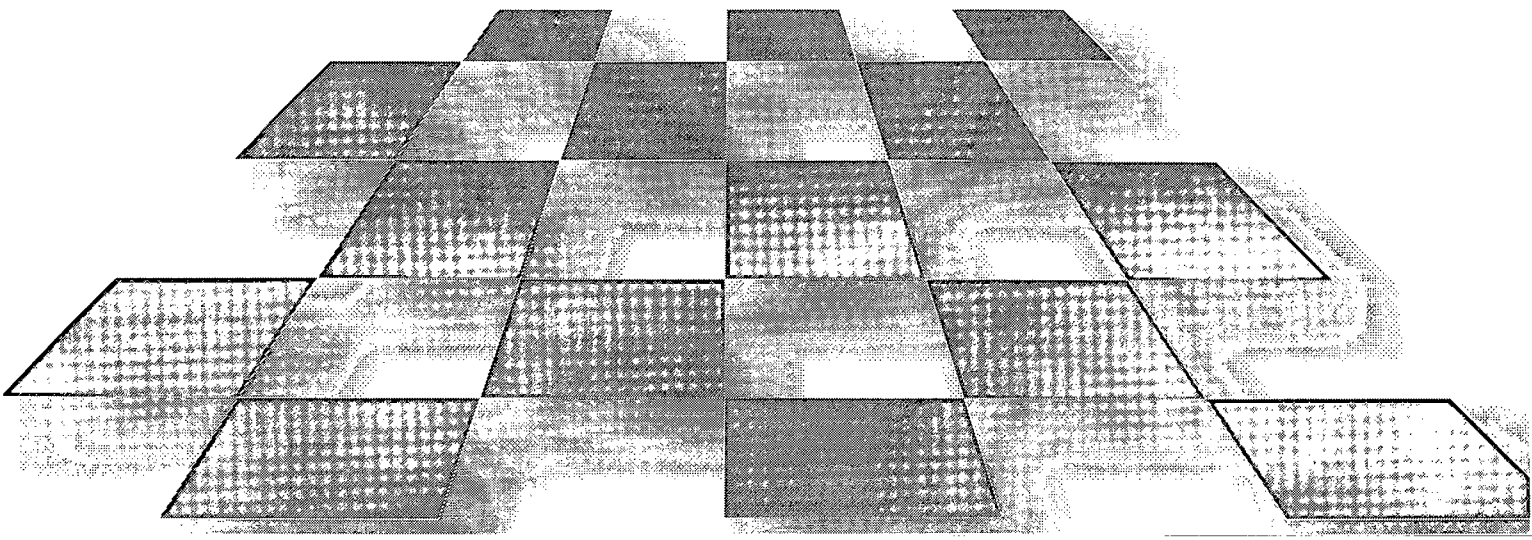
Study of methane production potential from soil using methanogenesis inhibitors

Soils from Site A and B at Lake Khajjiar were incubated with methyl fluoride-an inhibitor of acetoclastic methanogenesis to assess the hydrogenotrophic methanogenesis (Frenzel and Bosse, 1996). Soil slurries were prepared by mixing soil with autoclaved distilled water (under nitrogen atmosphere) in 1:3 (weight by volume) ratio. The slurry (20-25ml) was distributed in serum bottles (150ml), closed with rubber septa and then CH_3F was added in excess (of 1.5% by vol. of the headspace in the sample bottle). Three replicates of each soil depth (0-10, 10-15 and 15-20cm) were incubated at six different temperatures (4°C, 11°C, 15°C, 25°C, 37°C and 45°C). The head space gas namely methane, carbon dioxide and methyl fluoride was measured weekly for a month for site A and for a month and a half for site B. Steady state concentration of the hydrogen gas was measured at the end of the experiment

Statistical analysis

The data was analysed in Microsoft Office Excel 2000 and in Statistical Software-Statistical Package for Social Sciences 10.0.

RESULTS



Chapter IV

RESULTS

Methane Emission from Natural Wetland containing Peat Soil

Physicochemical characteristics of soil at Lake Khajjiar in 2005

The soil collected from the Lake Khajjiar was analysed for few basic parameters. The soil at Site-1 and 3 had circumneutral pH whereas at Site-2, the soil was relatively acidic (Table 2). The conductivity (mS) of soil was low at Site 2 compared to the other two sites (Table 2).

Table.2 The variation in pH and conductivity in soils at the three sites of Lake Khajjiar in 2005

Sites	pH		Conductivity	
	Mean	SD	Mean	SD
Site 1	7.1	0.1	6.0	0.8
Site 2	6.3	0.2	5.0	1.4
Site 3	7.1	0.6	5.9	1.5

Site-1 (Fig.5) showed a higher content of organic matter (%), Readily Mineralizable Carbon (RMC) and microbial biomass carbon across all three layers of soil core compared to the other two sites. The mean organic matter content was 63.61±9.22% at Site-1, 60.68±1.32% at Site-2 and 53.44±6.42 % at Site –3. At Site-1, the highest organic matter content was 70.75% from 0-5 cm core followed by 66.89% from 15-25 cm core and the minimum was 53.20% from the 5-15 cm core. At Site-2, the highest organic matter content was 64.59% from the 0-5cm, followed by 62.20 % and 59.97 % from 5-15 cm and 15-25 cm cores. At Site-3 the highest organic matter content was 60.70% from 0-5cm layer, followed by 59.69% in the 5-15cm and then 53.76% in 15-25cm layer. The RMC and microbial biomass content was highest at Site-1, followed by Site-2 and 3. The 0-5cm layer in each site showed higher RMC and microbial biomass carbon content. The Spearmann's nonparameteric correlation between % organic matter content and RMC is 0.619 and between % organic matter content and microbial biomass carbon is also 0.619.

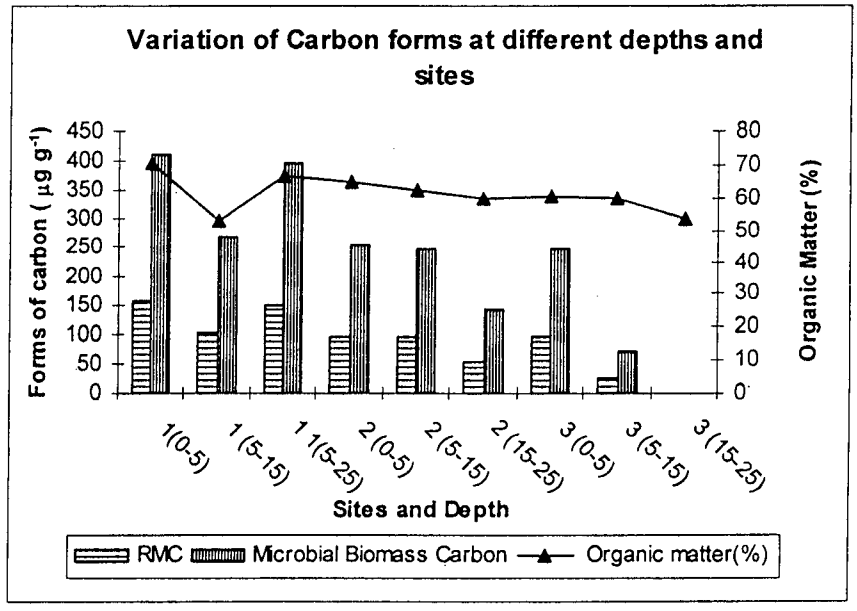


Fig.5 Variation of Readily Mineralizable Carbon (RMC) (µg g^{-1}), Microbial biomass carbon (µg g^{-1}) and organic matter (%) in different depths and sites in Khajjiar Lake in 2005

Methane Emission from dominant vegetation at Lake Khajjiar

Methane emission varied between the vegetated and unvegetated sediment, between different types of vegetation and also between the three sites (Fig.6).

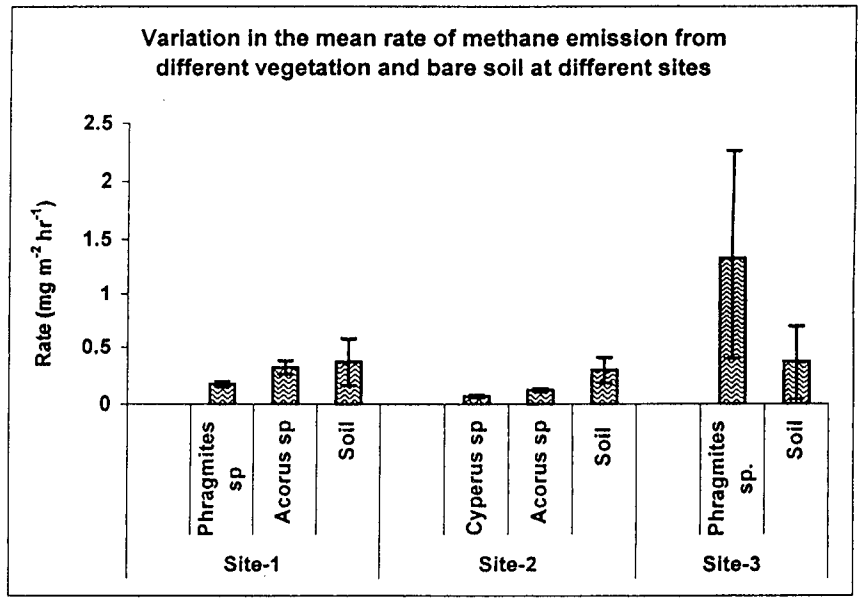


Fig.6 The variation in the average rate of methane emission (mg m^{-2} hr^{-1}) from unvegetated and vegetated soil at three different sites at Lake Khajjiar.

At Site-1 (Fig. 6), the bare soil emitted higher methane at rate of 0.37±0.21 $mgm^{-2}hr^{-1}$. Significant variation was not found between the three replicates of soil (F-value=2.03, F critical=5.14, p-value=0.212, df=8) and also between the flux on three days (F-values=0.658, F-critical values=5.14, p-value=0.55, df=8). The dominant plant species present in the site were *Phragmites* and *Acorus*. The rate of methane emission was 0.32±0.058 $mgm^{-2}hr^{-1}$from *Acorus* and 0.18±0.025 mg m^{-2} $hr^{-1}$from *Phragmites* sp. Significant variation in the rate of methane emission was not seen between the three replicates (F-value=4.495, F-critical value=5.14, p=0.05, df=8) and also between the emission over three days (F-value=0.67, F-critical value=5.143, p-value=0.54, df=8) from *Acorus* sp.

At Site–2 (Fig. 6), the rate of methane emission from bare soil was (0.30±0.11 mg $m^{-2}hr^{-1}$) higher than the vegetated soil. No significant variation was observed in the rate of methane emission between the soil replicates (F-value=1.33, F-critical value=5.14, P-value=0.33, df=8) and the flux over three days (F-value=1.01, F-critical value=5.14, P-value=0.42, df=8). Between the dominant plants present at this site the methane emission was higher from *Acorus* sp. (0.14±0.012 mg m^{-2} hr^{-1}) than *Cyperus* sp. (0.07±0.01). Significant variation in the rate of methane emission was not observed between the three replicates of *Acorus* sp. (F-value=0.36, F-critical value=5.14, P-value=0.71, df=8) and *Cyperus* sp. (F-value=0.11, F-critical value=5.14, P-value=0.89, df=8) but a significant variation was observed between the emissions over the three days in both the species (*Acorus* sp.- F-value=19.01, F-critical value=10.92, P-value=0.0025, df=8; *Cyperus* sp.-F-value=. 56.94, F-critical value=5.14, P-value=0.0001, df=8).

At Site–3 (Fig. 6), the vegetated soil showed higher rate of methane emission than the bare soil. The rate of methane emission from the dominant plant *Phragmites* sp. was 1.33±0.93 $mgm^{-2}hr^{-1}$and bare soil was 0.37±0.33 $mgm^{-2}hr^{-1}$. There was no significant variation in rate of methane emission from the replicates of *Phragmites* sp (F-value=4.7, F-critical value=5.14, P-value=0.059, df=8) and also between the emission over the three days (F-value=0.76, F-critical value=5.14, P-value=0.51, df=8). The emission of methane from bare soil did not differ significantly between the replicates (F-value=0.88, F-critical value=5.14, P-value=0.46, df=8) and also over the three days (F-value=0.79, F-critical value=5.14, P-value=0.49, df=8).

Inter-site variability in the rate of methane emission was not significantly different in soils (F-value=0.10, F-critical value=6.94, P-value=0.90, df=8 for rate of emission between three days at Site 1, 2 and 3; F-value=0.76, F-critical value=6.94, P-value=0.51, df=8 for rate of emission between soils at Site 1, 2 and 3 on each day). In plants, the maximum rate of methane emission was observed in *Phragmites* sp at Site 3 followed by *Acorus* sp in both the sites and the lowest rate was from *Cyperus* sp. A significant variation was observed between rates of emission from *Acorus* sp. at Site1 and Site 2 (F-value=18, F-critical value=7.70, P-value=0.012, df=5). A significant variation was also observed between rates of emission from *Phragmites* sp at Site 1 and Site 3 (F-value=7.74, F-critical value=7.70, P-value=0.049, df=5).

Ebullition or a very high rate of emission was observed from soil at a rate 17 mg m^{-2} hr^{-1} at Site 1 and 343 mg m^{-2} hr^{-1} at Site- 2.

Methane Emission and Plant biomass

The number of plants present during sampling and their corresponding biomass volume (cm^3), total leaf length (cm) and dry weight (g) are presented below.

Site-1

At Site-1 (Table 3), *Phragmites* plants were young and few in numbers compared to the Acorus plants. The plants in the first day were bit damaged while sampling, so the number of plants sampled in the first day varied from 2nd and 3rd day.

Table 3. The characteristics of the plants sampled from Site-1 at Lake Khajjiar.

Site 1				
	Phragmites	***Acorus* I**	***Acorus* II**	***Acorus* III**
No. of Plants				
Day -1	1	2	4	2
Day 2 & 3	1	3	5	3
Biomass Volume (cm^3)				
Day -1	14.27	54.13	135.07	54.76
Day 2 & 3	15.69	71.29	181.59	105.41
Total Leaf Length (cm)				
Day -1	171	536	918	668.5
Day 2 & 3	155.5	617	1137.5	778
Dry Weight (g)				
Day -1	2.17	5.84	14.06	8.69
Day 2 & 3	2.38	7.58	18.77	11.05

The regression equation and the r^2 value of the correlation between rate of methane emission (mg $m^{-2} hr^{-1}$) and biomass (gDW m^{-2}) of plants at Site –1 are given below (Fig.7).

Acorus 1: y= 0.0086x-0.3693, r^2=0.9776,

Acorus 2 : y= 0.0011x+0.1299, r^2=0.8039,

Acorus 3: y= 0.001x+0.0916, r^2=0.2474,

Phragmites sp. : y= -0.0151x+0.6045, r^2=0.7206

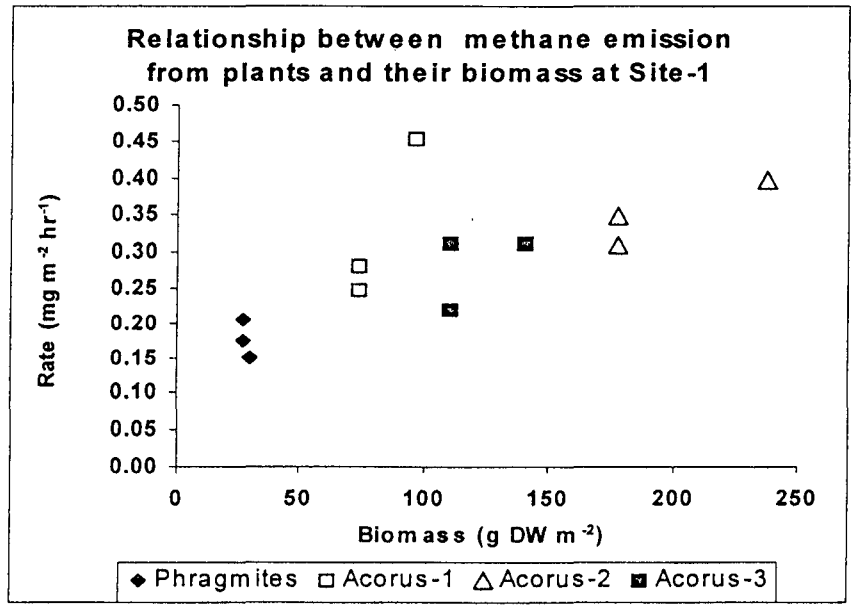


Fig.7 Variation in methane emission (mg $m^{-2} hr^{-1}$) from vegetations in relation to plant biomass (gDW m^{-2}) at Site-1.

The regression equation and the r^2 value of the correlation between rate of methane emission (mg $m^{-2} hr^{-1}$) and total shoot length (cm) of plants at Site-1 are given below (Fig. 8).

Acorus 1: y= 0.0002x-0.9964, r^2=0.9807

Acorus 2: y= 2E-05x+0.0492, r^2=0.8039

Acorus 3: y= 3E-05x-0.0279, r^2=0.2543

Phragmites sp. Y= -0.0002x+0.6045, r^2=0.7206

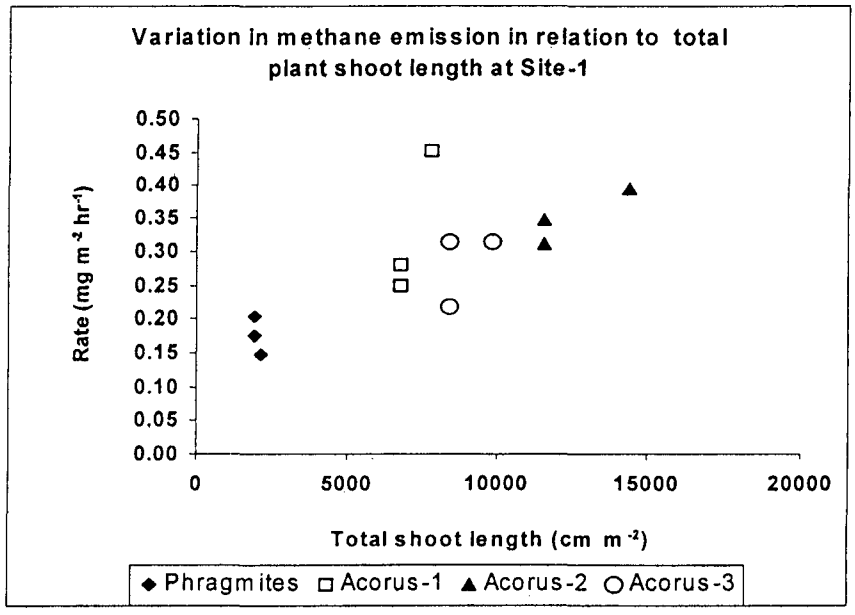


Fig.8 Variation in methane emission (mg $m^{-2} hr^{-1}$) from vegetations in relation to total shoot length (cm m^{-2}) in Site-1.

At Site-1, the rate of methane production did not change with an increase in the biomass (Fig. 7) and total shoot length (Fig. 8) of *Phragmites* sp., but it did increase in case of *Acorus* sp.

Site-2

The *Cyperus* plants (Table 4) have single culms and so there was variation in the shoot numbers between the sampling days and in *Acorus* sp. the vegetation was dense and so the shoots varied in the chambers in the first two days.

Table 4. The characteristics of the plants sampled from Site-2 at Lake Khajjiar.

Site-2						
	Cyperus-I	*Cyperus*-II	*Cyperus*-III	*Acorus*-I	*Acorus*-II	*Acorus*-III
No.of Plants						
Day –1	16	23	16	3	6	12
Day 2 & 3	14	21	16	4	8	13
Biomass Volume (cm^3)						
Day –1	67.64	75.32	63.88	128.29	161.77	288.91
Day 2 & 3	55.81	62.18	60.92	134.44	208.50	300.36
Total Leaf Length (cm)						
Day –1	855	952	807.5	886	1044	1644
Day 2 & 3	705.5	786	770	915	1264.5	1698
Dry Weight (g)						
Day –1	6.21	6.92	5.87	6.44	16.76	29.66
Day 2 & 3	5.12	5.71	5.59	13.99	21.50	30.82

The regression equation and the r^2 value of the correlation between rate of methane emission (mg $m^{-2} hr^{-1}$) and biomass (g DW m^{-2}) of plants at Site –2 are given below (Fig.9).

Cyperus 1 : y= -0.0002x+0.0439, r^2=0.0142

Cyperus 2 : y=0.0014x+0.0304, r^2=0.353

Cyperus 3 : y= -0.0006x+0.0747, r^2=0.0478

Acorus 1: y=0.0002x+0.0287, r^2=0.3332

Acorus 2: y=0.0011x-0.0935, r^2=0.9766

Acorus 3: y=0.0047x-1.5749, r^2=0.9948

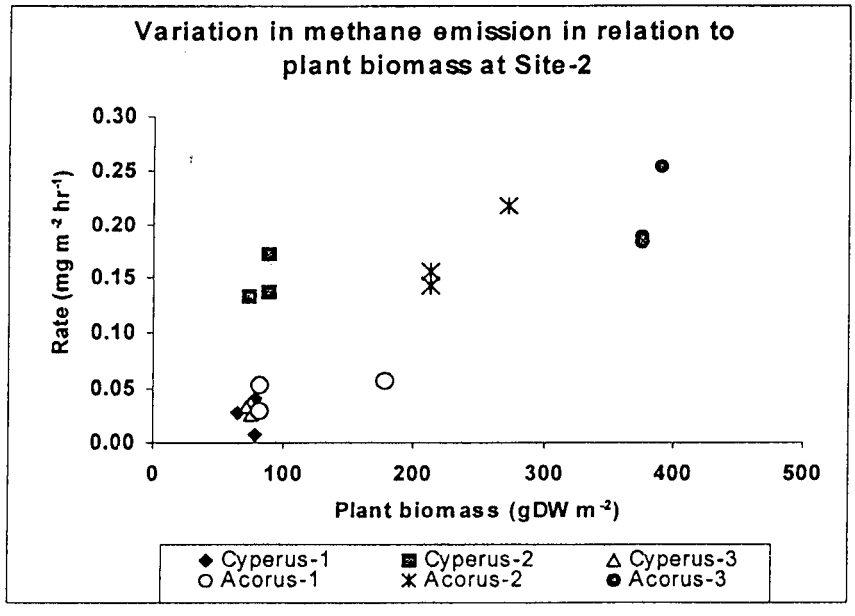


Fig.9 Variation in methane emission (mg $m^{-2} hr^{-1}$) from vegetations in relation to plant biomass (gDW m^{-2}) in Site-2.

The regression equation and the R^2 value of the correlation between rate of methane emission (mg $m^{-2} hr^{-1}$) and total shoot length (cm) of plants at Site-2 are given below (Fig.10).

Cyperus 1: y= -2E-05x+0.0439x, r^2= 0.0142

Cyperus 2 : y= 0.0001x+0.0304, r^2= 0.353

Cyperus 3: y= -5E-05x+0.0747, r^2= 0.0478

Acorus 1: y= 0.0005x-0.4096, r^2= 0.332

Acorus 2: y= 0.0003x-0.1755, r^2= 0.9766

Acorus 3: y= 0.0013x-1.912, r^2= 0.9947

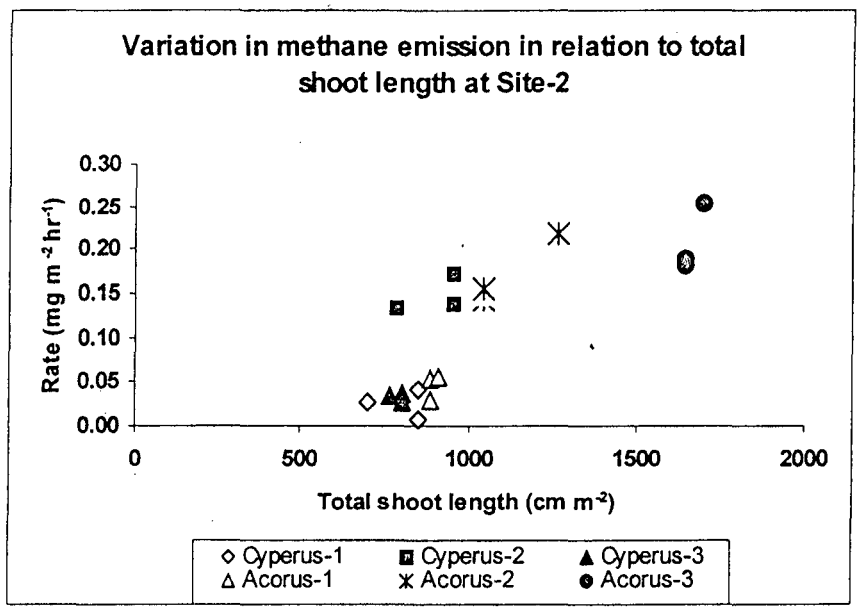


Fig.10 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to total shoot length (cm m^{-2}) at Site-2.

At Site-2 the rate of methane emission increased with the increase in total biomass (g DW m^{-2}) (Fig. 9) and total shoot length (cm m^{-2}) (Fig.10) .

Site-3

At Site-3 (Table 5), the plants and the sampling points were disturbed due to sampling and so the number of plants sampled in the last day varied.

Table 5: The characteristics of the plants that were sampled from Site-2 at Lake Khajjiar.

Site-3			
	Phragmites-I	**Phragmites-II**	**Phragmites-II**
No.of Plants			
Days 1 & 2	1	1	1
Day 3	1	1	1
Biomass Volume (cm^3)			
Days 1 & 2	15.23	18.53	15.05
Day 3	12.39	16.51	14.13
Total Leaf Length (cm)			
Days 1 & 2	166	202	164
Day 3	135	180	154
Dry Weight (g)			
Days 1 & 2	2.31	2.82	2.29
Day 3	1.88	2.51	2.15

The regression equation and the r^2 value of the correlation between rate of methane emission (mg m^{-2} hr^{-1}) and biomass (gDW m^{-2}) of plants at Site –3 are given below (Fig.11).

Phragmites 1: y= 0.0577x-0.4752, r^2= 0.1107

Phragmites 2 : y= 0.5121x-15.33, r^2= 0.8294

Phragmites 3 : y= 0.9225x-23.694, r^2= 0.9944

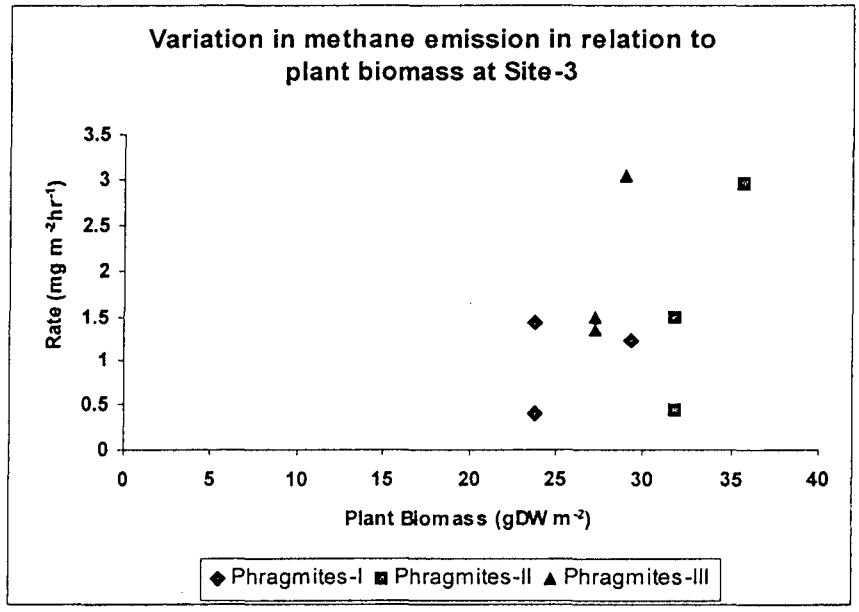


Fig. 11 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to plant biomass (gDW m^{-2}) at Site-3.

The regression equation and the r^2 value of the correlation between rate of methane emission (mg m^{-2} hr^{-1}) and total shoot length (cm) of plants at Site-3 are given below (Fig.12).

Phragmites 1: y=0.008x-0.4752, r^2=0.1107

Phragmites 2: y=0.0071x-15.33, r^2=0.82994

Phragmites 3 : y=0.0129x-23.694, r^2=0.9944

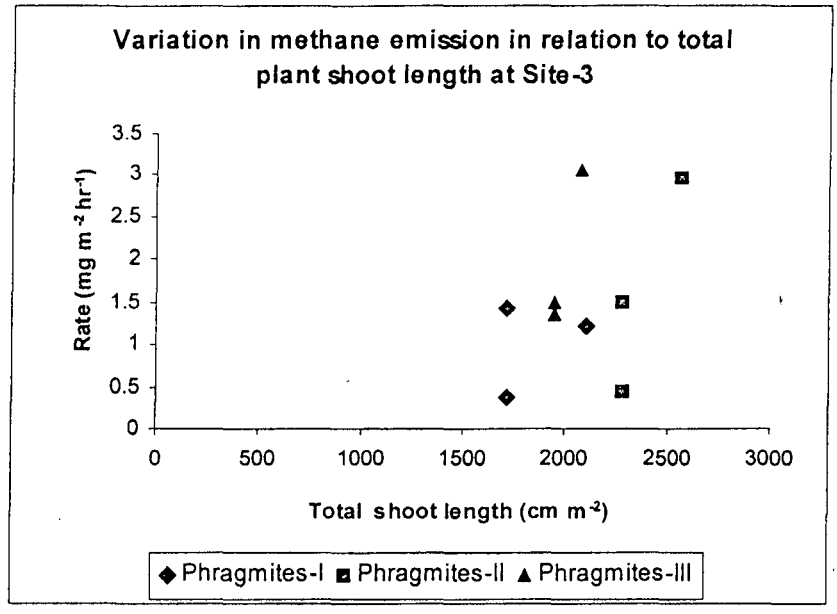


Fig.12 Variation in methane emission (mg m^{-2} hr^{-1}) from vegetations in relation to plant shoots (cm m^{-2}) at Site-3.

At Site-3 (Fig. 11) the rate of methane emission changed with or without the change in plant biomass (gDW m^{-2}) and total shoot length (Fig .12)

Methane Emission from Natural Wetland containing Mineral Soil

Experiments were conducted to investigate the role of plant in methane emission when grown in mineral soils. *Scirpus* plants were grown in soils of different types in tanks in the garden of School of Environmental Sciences.

Physicochemical factors of Soil

Texture analysis of soil (Table 6.) done at the end of the experiment showed that sand content of the Sandy soil was highest followed by Bhalswa soil and then Garden soil. The clay content of the Bhalswa soil was highest followed by Garden soil and then Sandy soil. The silt content of Garden soil was highest followed by Bhalswa soil and then Sandy soil.

Table 6. Texture analysis of soils used for growing *Scirpus* plants.

Soil Types	Sand %	Clay %	Silt %
Sandy soil	67.91 ± 3.93	14.84±0.44	19.75 ± 3.31
Bhalswa soil	45.03 ± 0.14	31.31 ± 0.66	23.67 ± 0.52
Garden soil	46.64 ± 1.97	20.2 ± 4.96	32.35 ± 3.87

The organic carbon determined by Walkley-Black method determines the partially oxidisable organic carbon of soil. The organic carbon content varied significantly across all soil types (F-value=10.77, F-critical value=4.25, p-value=0.004, df=11) but not over the four months (F-value=0.61, F-critical value=4.06, p-value=0.63,df=11) (Fig.13). It was high in Garden soil compared to Bhalswa and Sandy soil. The variation of organic carbon content over the four months was more pronounced in the Garden soil.

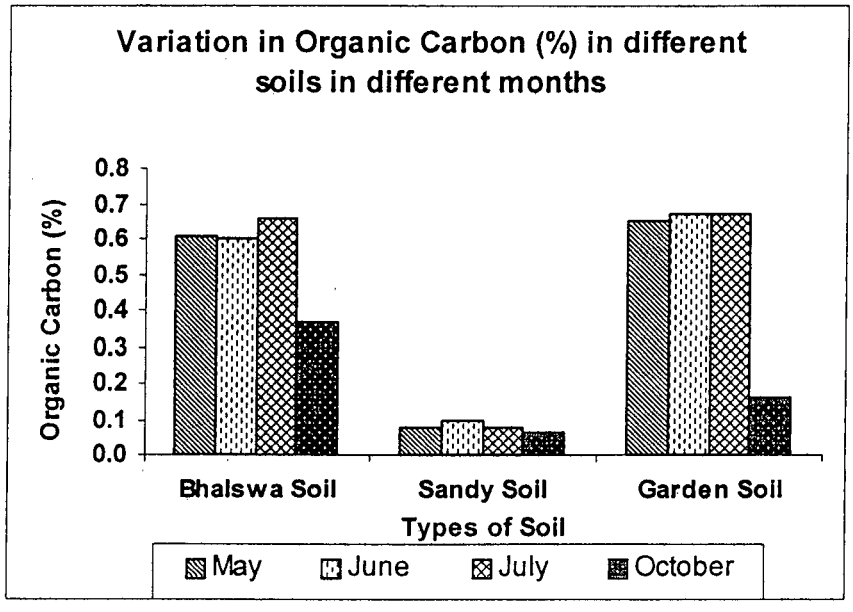


Fig.13 The variation in organic carbon content (%) of Bhalswa, Sandy and Garden soil over a period of four months.

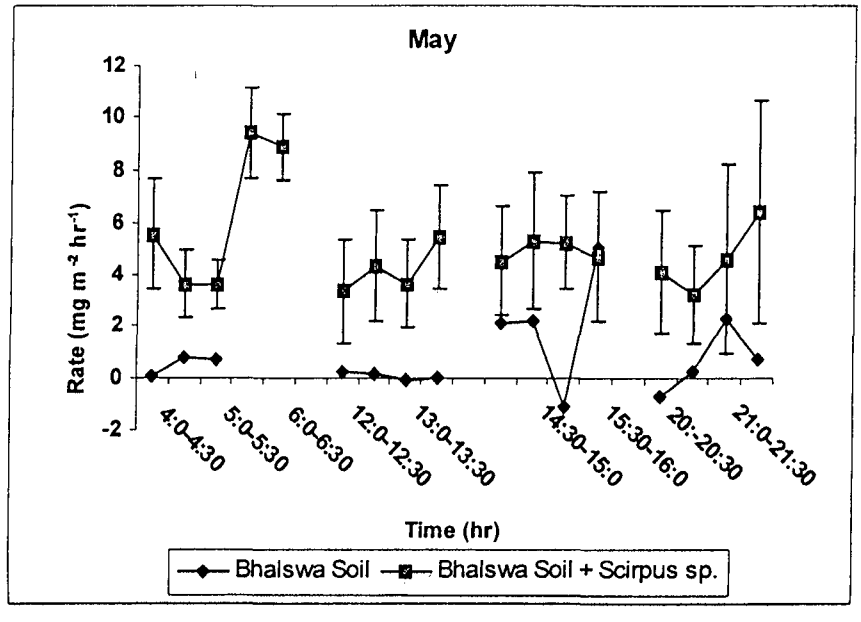
Total organic matter was analysed for the month of July by Loss on ignition method (Heiri et al, 2001) and total nitrogen by colorimetric method. The total organic matter and C/N ratio was higher in Bhlaswa soil, followed by Garden soil and Sandy soil (Table.7). The C/N ratio of Bhalswa soil was higher than Garden and Sandy soil.

Table 7. The variation in organic carbon (%), Total organic matter (%) and C/N ratio in three soil types in July.

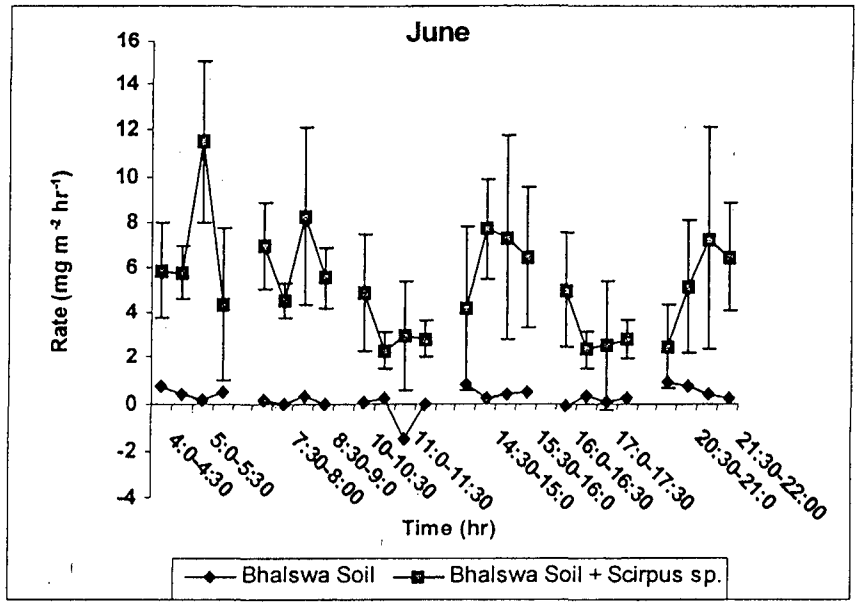
Soil Types	Organic Carbon (%)	Total organic matter (%)	Total Nitrogen (%)	C/N
Bhalswa Soil	0.48±0.01	7.53±0.05	0.17	2.82
Sandy Soil	0.065±0.01	2.25±0.38	0.27	0.24
Garden Soil	0.19±0.01	4.56±0.39	0.22	0.85

Methane emission from vegetated and bare soil of Bhalswa Lake

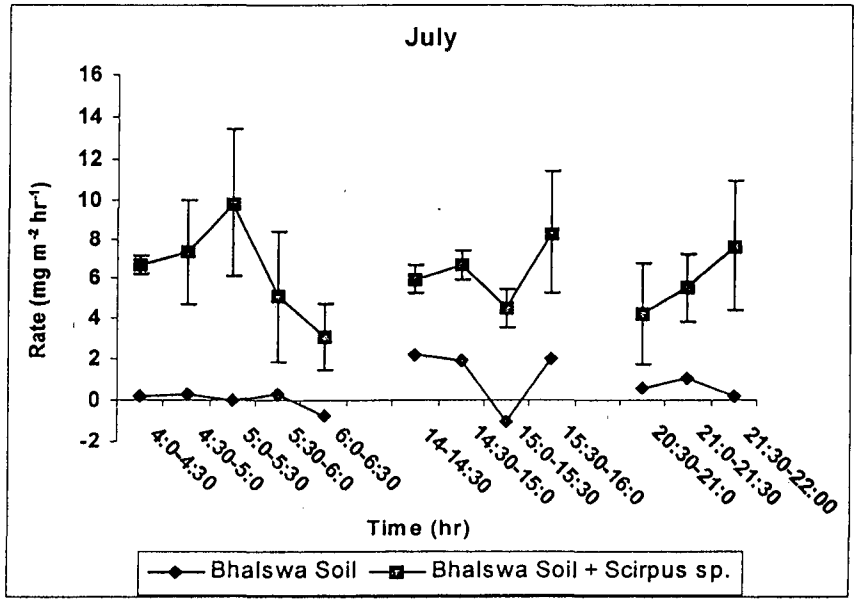
Methane emission from vegetated and bare soil of Bhalswa Lake was sampled in four different months. On 17th May, the samples were collected every half an hour for two hours. This sampling for two hour was repeated four times (4:00-6:00 hr, 12:00-14:00 hr, 14:00-16:00 hr and 20:00-22:00 hr) during the day. On 9 and 10th June, the frequency of sampling was increased to six times wherein the samples were collected for two hour from 4:00-6:00 hr, 7:00-9:00 hr, 11:00-13:00 hr, 14:00-16:00 hr, and 20:00–22:00 hr on the first day and from 16:00-18:00 hr in the consecutive day. On 2nd July and 5th October, sampling was done for three times covering day and night- 4:00-6:30 hr, 12:00-14:00 hr and 20:00-22:00 hr.



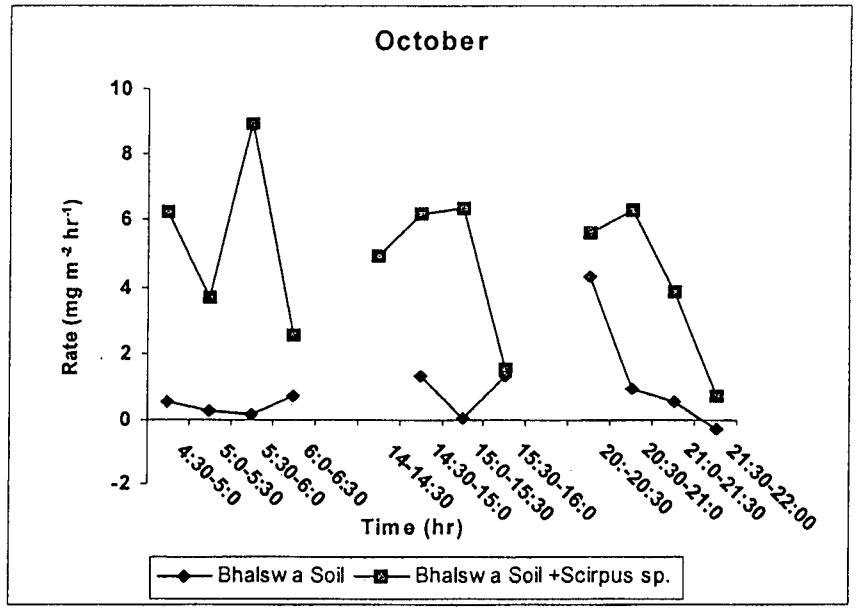
a)


June
Rate (mg m-2 hr-1)
16
14
12
10
8
6
4
2
0
-2
-4
4:0-4:30
5:0-5:30
7:30-8:00
8:30-9:0
10-10:30
11:0-11:30
14:30-15:0
15:30-16:0
16:0-16:30
17:0-17:30
20:30-21:0
21:30-22:00
Time (hr)
Bhalswa Soil
Bhalswa Soil + Scirpus sp.

b)


July
Rate (mg m-2 hr-1)
16
14
12
10
8
6
4
2
0
-2
4:0-4:30
4:30-5:0
5:0-5:30
5:30-6:0
6:0-6:30
14-14:30
14:30-15:0
15:0-15:30
15:30-16:0
20:30-21:0
21:0-21:30
21:30-22:00
Time (hr)
Bhalswa Soil
Bhalswa Soil + Scirpus sp.

c)



d)

Fig.14. Variation in rate of methane emission (mg m^{-2} hr^{-1}) for every half an hour interval, from bare and vegetated Bhalswa soil a) on 17th May 2006, b) 9th and 10th June 2006, c) 2nd July 2006 and d) 5th October 2006

The result of the methane sampling was the following:-

1) The rate of methane emission from vegetated soil was always higher than the bare soil
2) The peak emission was always after sunrise and as the time of sunrise shifted from month to month, the timing of the peak rate of emission shifted over the months.

On 17th May (Fig.14a), the rate of methane emission fluctuated more in vegetated sediments and was higher (ranged from 3.19±1.91 to 9.38±1.75 mg m^{-2} hr^{-1}) than the unvegetated sediment (ranged from -1.11 to 2.25 mg m^{-2} hr^{-1}) of Bhalswa soil. The rate of methane emission for every half an hour did not vary but a distinct peak between 5:30-6:00 hr at a rate of 9.38±1.75 mg m^{-2} $hr^{-1}$was observed in the early morning after sunrise. The rate of methane emission showed a common trend of increase for every half an hour within the two hours. In the noon and afternoon from 12:00 to 14:00 hr and 14:00 to 16:00 hr a stable rate of methane emission was observed. The rate of methane emission at night from 20:00 to 22:00 hr, increased from 4.09±2.38 to 6.37±4.29 mg m^{-2} hr^{-1}. The rate of methane emission ranged from 0.49 to 0.76 mg m^{-2} hr^{-1}between 4:00-6:30 hr, -1.11 to 5.00 mg m^{-2} hr^{-1} between

14:00-16:00 hr and –0.76 to 0.65 mg $m^{-2} hr^{-1}$ between 20:00-22:00 hr. The sudden rise in the rate of emission from bare soil at 15:30 hr at a rate of 5.00 mg $m^{-2} hr^{-1}$ may be due to ebullition.

On 9th and 10th June (Fig.14b), the frequency of sampling was increased. The rate of methane emission varied significantly in every two hours over the six sampling time points (F-value=7.35, F-critical value=3.33, P value=0.004, df=17). The rate of emission varied from 2.32±0.832 to 11.53±3.547 mg m^{-2} hr^{-1} in the vegetated sediment. In the vegetated sediment the highest peak (11.53 ±3.547 mg $m^{-2} hr^{-1}$) was observed between 5:00-5:30 hr. The rate of emission dipped at 10:00-12:00 hr (from 4.50±2.60 to 2.85±0.85 mg $m^{-2} hr^{-1}$) and 16:00-18:00 hr (from 5.01±2.57 to 2.79±0.88 mg $m^{-2} hr^{-1}$). The rate of emission increased at night from 20:00-22:00 hr increased from 2.50±1.87 to 7.23±4.86 mg $m^{-2} hr^{-1}$till 21:30 hr and then declined in the last half an hour to 6.46±2.35 mg $m^{-2} hr^{-1}$. The rate of emission recorded last at 22:00 hr was close to the rate of emission (5.86±2.14 mg $m^{-2} hr^{-1}$) seen in the dark hours before dawn (4:00-5:00 hr). The rate of emission from bare soil was low and consistent over different time of day and night. The rate of methane emission varied from the unvegetated soil ranged from 0.10 to 0.71 mg $m^{-2} hr^{-1}$between 4:00-6:00 hr, -0.07 to 0.33 mg $m^{-2} hr^{-1}$between 7:00-9:00 hr, -1.48 to 0.24 mg $m^{-2} hr^{-1}$ between 10:00-12:00 hr, 0.20 to 0.82 mg $m^{-2} hr^{-1}$between 14:00-16:00 hr, -0.09 to 0.34 mg $m^{-2} hr^{-1}$ between 16:00-18:00 hr and 0.26-0.91 mg $m^{-2} hr^{-1}$between 20:00-22:00 hr.

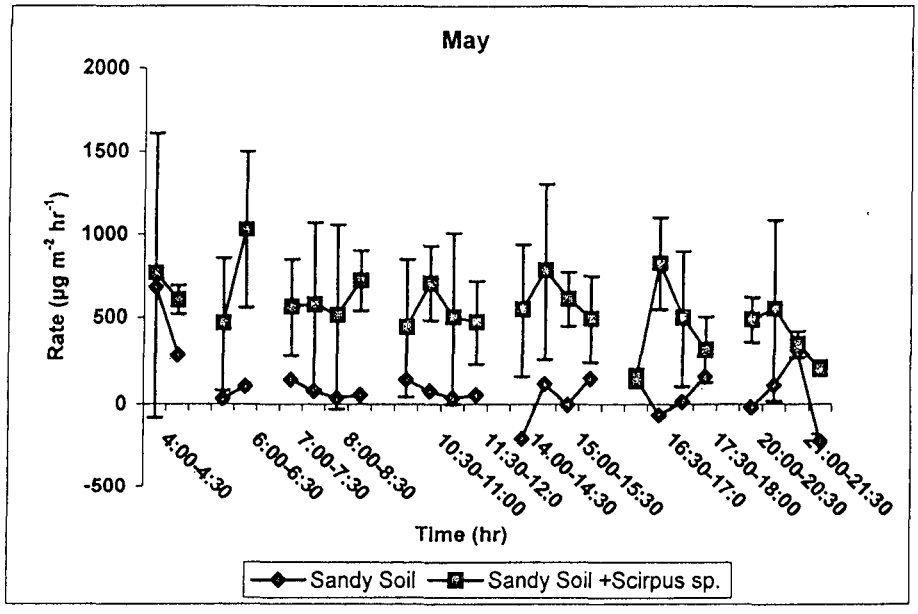
On 2nd July (Fig.14c), the rates did not vary significantly in every half an hour over the three sampling period (F-value=5.68, F-critical value=6.94, P-value=0.068, df=2). The peak emission was distinct between 5:00-5:30 hr at a rate of 9.77±3.65 mg $m^{-2} hr^{-1}$. The rate of emission declined to 3.08±1.61 mg m^{-2} hr^{-1} (minimum observed) between 6:00-6:30 hr after the peak emission. Between 14:00 and 16:00 hr the rate of emission increased till 15:00 hr at a rate of 5.95±0.69 to 6.69±0.75 mg $m^{-2} hr^{-1}$, dipped at 15:30 hr at a rate of 4.47±0.92 mg $m^{-2} hr^{-1}$and then shot up to 8.31±3.09 mg $m^{-2} hr^{-1}$. At night between 20:30 and 22:00 hr the rate of methane emission increased from 4.23±2.51 to 7.66±3.292 mg $m^{-2} hr^{-1}$. In the early hours of dawn before the sunrise, the rate of emission ranged from 6.65±0.47 to 7.33±2.64 mg $m^{-2} hr^{-1}$. In the bare soil the rate of methane emission ranged from –0.79 to 0.26 mg $m^{-2} hr^{-1}$ between 4-6:30 hr,

-1.06 to 2.16 mg $m^{-2} hr^{-1}$ between 14:00-16:00 hr and 0.22 to 1.05 mg $m^{-2} hr^{-1}$between 20:00-22:00 hr.

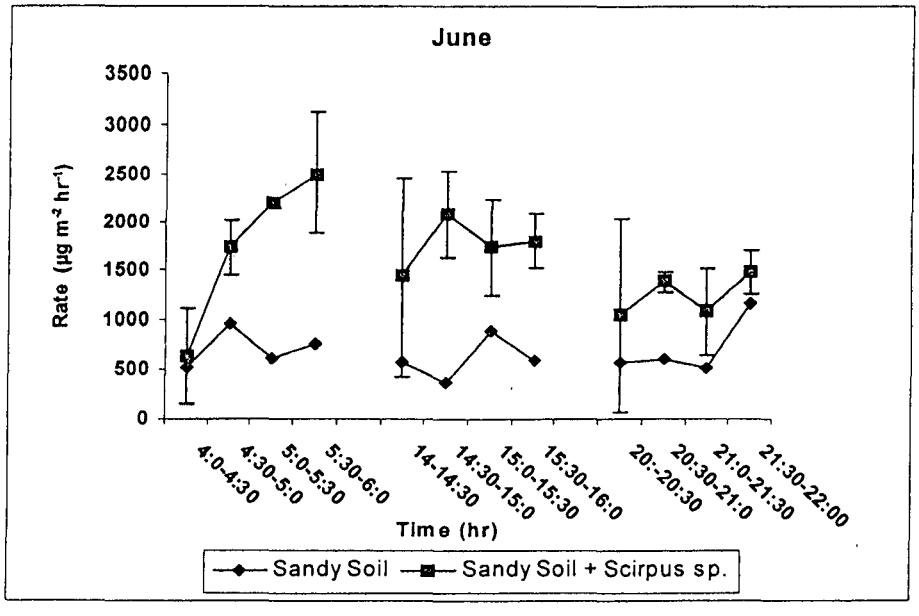
On 5th October (Fig.14d), the rate methane emission was sampled from *Scirpus* plants grown in two tanks only. The peak emission was distinct after sunrise between 5:30-6:00 hr at a rate of 8.94 mg $m^{-2} hr^{-1}$. The rate of emission declined to 2.59 mg $m^{-2} hr^{-1}$after the peak. In the afternoon the rate of emission increased (4.94 to 6.32 mg $m^{-2} hr^{-1}$) every half an hour till 3:30 hr and then the rate declined to 1.57 mg $m^{-2} hr^{-1}$. At night between 20:00 and 22:00 hr the rate increased from 5.62 to 6.27 mg $m^{-2} hr^{-1}$ till 21:00 hr and then decreased gradually to 3.86 and 0.73 mg $m^{-2} hr^{-1}$ (minimum peak observed) between 21:30 to 22:00 hr. In the bare soil the rate of emission varied from -0.31 to 4.33 mg $m^{-2} hr^{-1}$. The rate in the early morning before sunrise was at 6.23 to 3.68 mg $m^{-2} hr^{-1}$ between 4:30-5:30 hr. In the bare soil the rate of methane emission ranged from 0.13 to 0.70 mg $m^{-2} hr^{-1}$ between 4:00-6:30 hr, 0.039 to 1.328 mg $m^{-2} hr^{-1}$ between 14:30-16:00 hr and –0.308-4.325 mg $m^{-2} hr^{-1}$ between 20:00-22:00 hr.

Methane emission from vegetated and bare Sandy Soil

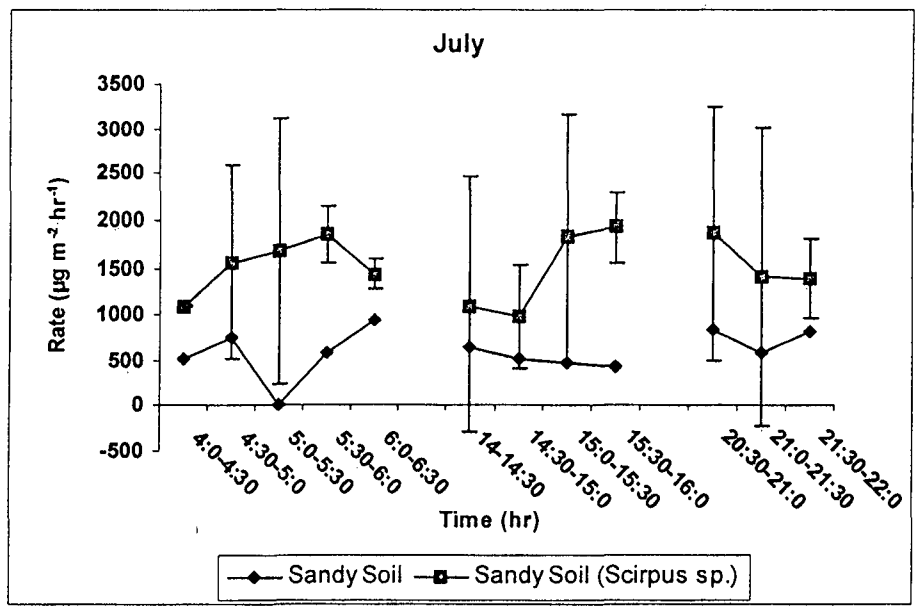
In 10th and 11th May, the plants and bare soil were sampled 6 times for every half an hour between 4:00-6:30 hr, 7:00-9:00 hr, 10:00-12:00 hr, 14:00-16:00 hr, 16:00-18:00 hr and 20:00-22:00 hr to estimate the diurnal rate of emission. The sampling point of 16:00-18:00 hr was conducted in the consecutive day. On 14th June, 1st July and 10th October the sampling was done three times for every half an hour between 4:00-6:30 hr, 14:00-16:00 hr and 20:00-22:00 hr.


May
2000
1500
1000
500
0
-500
Rate (µg m-2 hr-1)
4:00-4:30
6:00-6:30
7:00-7:30
8:00-8:30
10:30-11:00
11:30-12:0
14:00-14:30
15:00-15:30
16:30-17:0
17:30-18:00
20:00-20:30
21:00-21:30
Time (hr)
Sandy Soil
Sandy Soil +Scirpus sp.

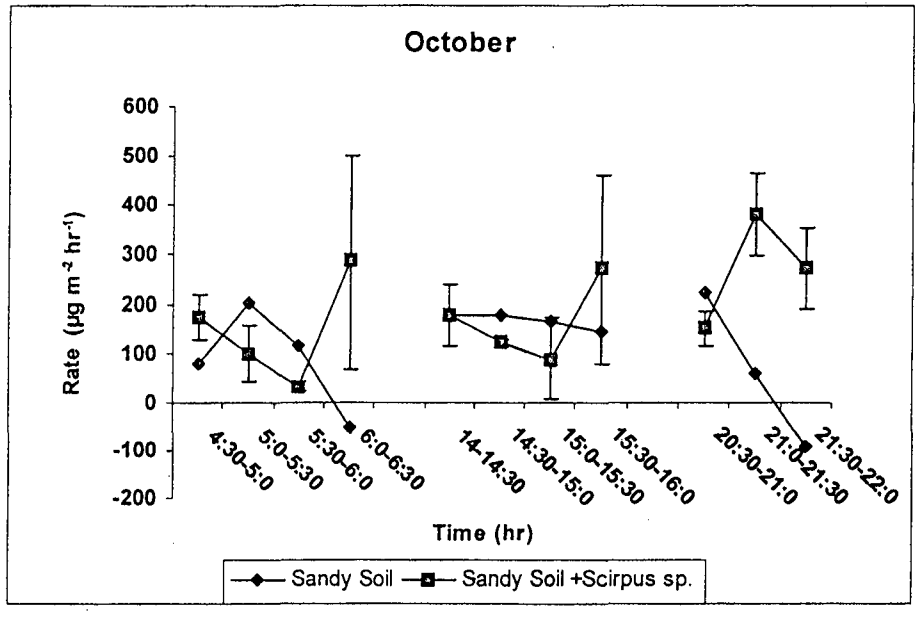
a)


June
3500
3000
2500
2000
1500
1000
500
0
Rate (µg m-2 hr-1)
4:0-4:30
4:30-5:0
5:0-5:30
5:30-6:0
14-14:30
14:30-15:0
15:0-15:30
15:30-16:0
20:-20:30
20:30-21:0
21:0-21:30
21:30-22:00
Time (hr)
Sandy Soil
Sandy Soil + Scirpus sp.

b)



c)



d)

Fig.15 Variation in rate of methane emission (µg m^{-2} hr^{-1}) for every half hour an interval, from bare and vegetated Sandy soil on a) 10th and 11th May 2006, b) 14th June 2006, c) 1st July 2006 and d) on 10th October 2006

The result of the methane sampling was the following:-

1) The rate of methane emission was higher from the vegetated sediment compared to the unvegetated sediment except in October when the emission from soil sometimes was more than the former.

2) The rate of emission always peaked after the sunrise. The peak shifted with the shift in time of sunrise.
3) The rate of methane emission was low (expressed in terms of $\mu g\ m^{-2}\ hr^{-1}$) compared to the Bhalswa and Garden soil.

On 10th and 11th May (Fig.15a), the rate of methane emission ranged from 2504.86 ± 622.98 to 629.00 ± 483.59$\mu g\ m^{-2} hr^{-1}$ from the vegetated sediment. Methane emission peaked at a rate of 1029.70±471.23 $\mu g\ m^{-2} hr^{-1}$ in between 5:30 and 6:00 hr. The rate of emission ranged from 564.44±283.42 to 578.54±494.39 $\mu g\ m^{-2} hr^{-1}$ between 7:00 and 8:00 hr, it declined to 515.72±543.81 $\mu g\ m^{-2} hr^{-1}$ between 8:00 and 8:30 hr and increased to 719.83±180.66 $\mu g\ m^{-2} hr^{-1}$ between 8:30 and 9:00 hr. Between 10:00 and 12:00 hr, the rate fluctuated every half hour. The rate of emission increased from 549.70±390.06 to 783.20±523.71 $\mu g\ m^{-2} hr^{-1}$ untill 15:00 hr and then declined to 493.64±252.45 $\mu g\ m^{-2} hr^{-1}$ between 15:30 and 16:00 hr. In the evening between 16:00 and 18:00 hr, the trend of the rate of emission was similar to that in afternoon. At night too the trend was same but the rate dipped to 208.55±43.65 $\mu g\ m^{-2} hr^{-1}$. The mean rate of 764.99±942.92 $\mu g\ m^{-2} hr^{-1}$ (methane emission) observed at the first hour of sampling before dawn is close to the rate observed between 21:30 and 22:00 hr. The rate of emission did not vary significantly for every half an hour for the six time points (F-value=0.55, F-critical value=2.99, P-value=0.76, df=14). The rate of methane emission in the unvegetated sediment ranged from 367-1169.67 $\mu g\ m^{-2} hr^{-1}$.

On 14th June (Fig.15b), the rate of methane emission ranged from 629±483.59 $\mu g\ m^{-2} hr^{-1}$ to 2504±622.98 $\mu g\ m^{-2} hr^{-1}$. The peak rate of emission was 2504±622.98 $\mu g\ m^{-2} hr^{-1}$ between 5:30 and 6:00 hr. In the afternoon (14:00-16:00 hr), the rate of methane emission increased from 1452±1018.54 $\mu g\ m^{-2} hr^{-1}$ to 2095.73±460.21 $\mu g\ m^{-2} hr^{-1}$ in the first one hour and then decreased slightly to 1746.11±496.42$\mu g\ m^{-2} hr^{-1}$ to increase again to 1804.29±290.51 $\mu g\ m^{-2} hr^{-1}$ in the next one hour. At night, the rate fluctuated between 1053.0±974.21to 1478.49±226.27 $\mu g\ m^{-2} hr^{-1}$. The rate observed between 21:30 and 22:00 hr was higher than the rate of emission (629±483.59 $\mu g\ m^{-2} hr^{-1}$) observed first in the early dawn before sunrise. But the rate of emission did increase to 1740±26.54 and 2199.10±58.20 $\mu g\ m^{-2} hr^{-1}$ in the next one hour before the final peak after sunrise.

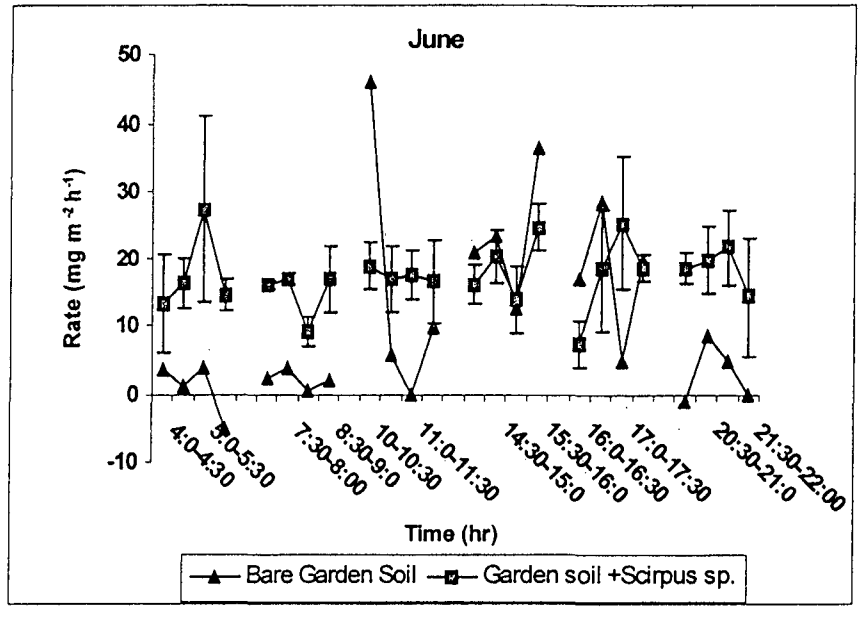
The rate of emission in the unvegetated sediment ranged from 511-1169.67 μg m^{-2} hr^{-1}. The sudden increase to 1169 μg m^{-2} hr^{-1} between 21:30 and 22:00 hr from bare soil may be due to ebúllition.

On 1st July (Fig.15c), in the vegetated sediment, the rate of methane emission ranged from 1852±295.51 to 978.82±559.43 μg m^{-2} hr^{-1}. The peak rate of emission was between 5:30 and 6:00 hr and then the rate declined to 1432.43±156.87μg m^{-2} hr^{-1} in the next half an hour. In afternoon, the rate of methane emission fluctuated from 1093.95±1379.27 μg m^{-2} hr^{-1} to 978.82±559.43 μg m^{-2} hr^{-1} in the first one hour. The rate then increased to 1931.88±371.53 μg m^{-2} hr^{-1} within an hour. At night the rate of emission declined from 1864.00±1379.00 μg m^{-2} hr^{-1} to 1386.89±422.82 μg m^{-2} hr^{-1} between 20:30 and 21:00 hr. The rate found at the early hour increased from 1093.80±16.82 μg m^{-2} hr^{-1} to 1679.09±1432.98 μg m^{-2} hr^{-1} before shooting to the peak rate of methane emission. In the unvegetated sediment the rate of methane emission ranged from 4.13 to 933.74 μg m^{-2} hr^{-1}. The rate of methane emission fluctuated highly from 4:00-6:30 hr in the unvegetated sediment. On average, the rate of methane emission for every half an hour did not vary for the other two sampling points.

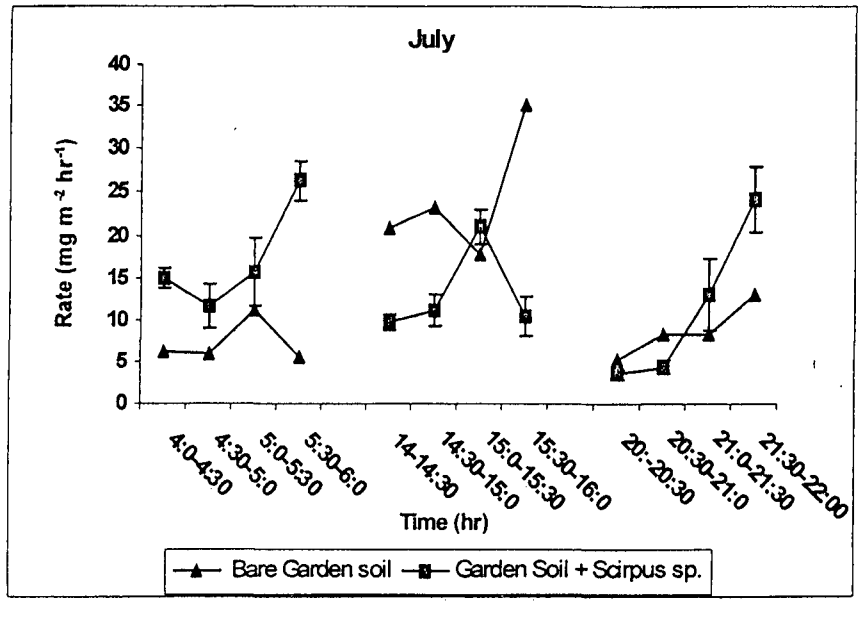
On 10th October (Fig.15d), the rate of emission was very low. In the vegetated sediment it ranged from 34.527±10.52 μg m^{-2} hr^{-1} to 285.43±240.35 μg m^{-2} hr^{-1}. A small peak (285.43±214.76 μg m^{-2} hr^{-1}) was observed between 6:00 and 6:30 hr. In the afternoon the rate fluctuated from 177.74±61.64 μg m^{-2} hr^{-1} to 91.24±81.20 μg m^{-2} hr^{-1} within one and a half hour and then the rate increased to 272.109±189 μg m^{-2} hr^{-1} in the last half hour. At night from 20:30 to 22:00 hr the rate fluctuated between 305.40±100.64 μg m^{-2} hr^{-1} and 270.82±80.15 μg m^{-2} hr^{-1}. The rate of emission ranged from 173.96±42.49 to 34.53±9.69 μg m^{-2} hr^{-1} before the peak rate of emission after sunrise. The rate of emission in the unvegetated sediment ranged from –92.32 to 222.69 μg m^{-2} hr^{-1}. In the afternoon the rate of methane emission was higher from the vegetated than the bare soil except in the last half an hour when the rate of emission from the vegetated sediment shot up. The rate of methane emission declined steeply to –92.32 μg m^{-2} hr^{-1} at night.

Methane Emission from vegetated and bare Garden Soil

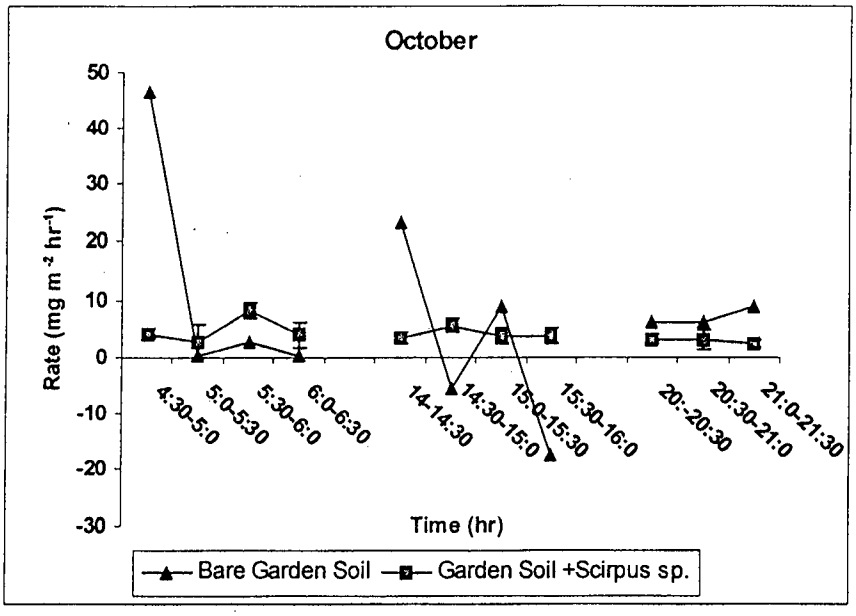
The plants grown on garden soil were sampled along with unvegetated garden soil. On 5th and 6th June, the sampling was conducted six times over two consecutive days. The samples were collected every half an hour from 4:00-6:00 hr, 7:00-9:00 hr, 10:00-12:00 hr, 14:00-16:00 hr, 16:00-18:00 hr and 20:00-22:00 hr. On 7th July and 10th October, the time points of sampling were 4:00-6:00 hr, 14:00-16:00 hr and 20:00-22:00 hr. The highest peak of rate of emission was observed to be always after sunrise.



a)



b)



c)

Fig.16. Variation in rate of methane emission (mg m^{-2} hr^{-1}) for every half an hour interval from bare and vegetated garden soil on a) 5th and 6th June 2006, b) 7th July 2006, c) 12th October 2006

On 5th and 6th June (Fig.16a), in the vegetated Garden soil, the rate of methane emission ranged from 285.42±214.76 to 34.53±4.69 mg m^{-2} hr^{-1}. The peak rate of methane emission 34.53±4.69 mg m^{-2} hr^{-1} was observed between 5:00 and 5:30 hr after sunrise. In the morning between 7:00 and 9:00 hr, the rate increased from 15.79±0.65 to 16.80±1.02 mg m^{-2} hr^{-1}. In noon from 10:00-12:00 hr the rate fluctuated between 18.75±3.67 and 16.56±6.20 mg m^{-2} hr^{-1}. In the afternoon from 14:00-16:00 hr, the rate increased from 15.94±3.0 to 20.09±4.06 in the first hour and decreased to 13.63±4.92 mg m^{-2} hr^{-1} to again increase to 24.55±3.61 mg m^{-2} hr^{-1}. In the evening, from 16:00-18:00 hr, the rate increased steadily from 7.18±3.41 to 25.22 ± 10.09 mg m^{-2} hr^{-1} and then decreased to 18.35±1.97 mg m^{-2} hr^{-1} in the last half hour. At night between 20:00 and 21:30 hr, the rate of methane emission increased from 18.44±2.33 to 21.59±5.64 mg m^{-2} hr^{-1} and then declined to 14.20±8.72 mg m^{-2} hr^{-1}. The rate observed between 20:30 and 21:00 hr (14.09±9.67 mg $m^{-2}hr^{-1}$) was close to the rate in the first hour 4:30 and 5:00 hr (16.09±4.05 mg m^{-2} hr^{-1}) in the morning before the peak rate of emission. In the unvegetated sediment the rate of emission ranged from –1.09 to 45.95 mg m^{-2} hr^{-1}. The rate was stable between 4:00-6:00 hr, 7:00-9:00 hr and 20:00-22:00. The high rate of methane emission that was observed between 10:00 and

10:30 hr (45.95 mg $m^{-2} hr^{-1}$) could be due to ebullition. In the afternoon from 14:00-16:00 and 16:00-18:00 hr the rate of emission from bare soil was found to be higher than from the vegetated soil.

On 1st July (Fig.16b), the rate of methane emission ranged from 3.44±0.49 to 26.22± 2.27 mg $m^{-2} hr^{-1}$ in the vegetated sediment. The peak was observed between 5:30-6:00 hr after sunrise. In the afternoon, from 14:00-16:00 hr, the rate increased gradually from 3.44±0.49 to 20.99±2.08 mg $m^{-2} hr^{-1}$ and then decreased to 10.44±2.33 mg $m^{-2} hr^{-1}$ in the last half an hour. At night, the rate increased gradually from 3.44±0.49 to 24.20±3.80 mg $m^{-2} hr^{-1}$ till 22:00 hr. In the early dawn (from 4:00-5:00 hr) before sunrise, the rate of methane emission ranged between 14.92±1.2 to 15.66±4.02 mg $m^{-2} hr^{-1}$.

The rate of methane emission from the unvegetated sediment ranged from 4.05 to 35.10 mg $m^{-2} hr^{-1}$. The rate of emission from bare soil shoots up to 35.10 mg $m^{-2} hr^{-1}$ in the afternoon. The rate of emission from bare soil is higher compared to the vegetated soil in afternoon and in the first hour of evening.

On 10th October (Fig.16c), the rate of methane emission from the vegetated sediment ranged from 2.23±0.71 to 8.10±1.67 mg $m^{-2} hr^{-1}$. The peak rate of emission was observed between 5:30 and 6:00 hr after sunrise. The rate of emission increased from 3.26±1.09 mg $m^{-2} hr^{-1}$ in the first hour of afternoon to 5.49±1.24 mg $m^{-2} hr^{-1}$ between 14:30 and 15 hr and then decreased to 3.58±1.48 mg $m^{-2} hr^{-1}$ in the last hour between 15:30-16:00 hr. At night the rate declined from 3.07±1.07 to 2.23±0.73 mg $m^{-2} hr^{-1}$between 20:00-21:30 hr. In the first one hour of sampling before sunrise the rate of emission ranged from 4.14±0.86 to 2.77±3.30 mg $m^{-2} hr^{-1}$. The rate of methane emission from the unvegetated soil ranged from –17.50 –46.57 mg $m^{-2} hr^{-1}$. The rate of emission of methane from bare soil varied with time of the day. In the early morning from 4:00-4:30 hr there was an ebullition of 45.67 mg $m^{-2} hr^{-1}$. In the afternoon the rate of emission was high 23.38 mg $m^{-2} hr^{-1}$ in the first half an hour between 14-14:30 hr and then dipped to –5.59 mg $m^{-2} hr^{-1}$ in the next half an hour between 14:30–15:00 hr. The rate again increased to 8.69 mg $m^{-2} hr^{-1}$ and dipped to –17.50 mg $m^{-2} hr^{-1}$. At night the rate of methane emission was stable around 6.00 mg $m^{-2} hr^{-1}$.

Total Methane Emission

To calculate the total methane emission (Table.8) for a single day mean rates were calculated (from every half an hour) for every two hours observation for which the measurements were done and multiplied with the number of hours of measurement i.e two hours. For the gaps in which sampling was not done, the average rate was worked out from the last half hour rate of emission of the previous observation and the first half hour rate of emission of the next observation between two sampling time points and multiplied it with the number of in between hours. The integration of all individual mean hourly emissions led to the total emission for that day. The total methane emission from the *Scirpus* plants of Bhalswa Soil was nearly consistent except in October. The total methane emission from the Scirpus plants of Sandy soil was highest in June and reduced thereafter. The total methane emission from the *Scirpus* plants of Garden soil was highest in July and reduced thereafter. The total methane emission from unvegetated Sandy soil and Garden soil was nearly consistent over the three months but fluctuated in Bhalswa Soil.

Table 8. Total methane emission (mg m^{-2}) from vegetated and bare soil in different month.

Months	Bhalswa Soil		Sandy Soil		Garden Soil	
	Vegetated Soil	Bare Soil	Vegetated Soil	Bare Soil	Vegetated Soil	Bare Soil
	Mean±SD n=3 (mg m^{-2})	Mean (mgm^{-2})	Mean±SD n=3 (mg m^{-2})	Mean (mg m^{-2})	Mean±SD n=3 (mg m^{-2})	Mean (mg m^{-2})
May	129.07±54.11	18.11	11.46±1.96	2.73	-	-
June	119.04±44.37	7.97	37.39±11.59	16.79	841±14.92	202.27
July	129.09±29.87	43.50	34.49±16	16.30	377.18±20.90	269.36
October	93.62±9.54	22.51	5.17±1.80	1.89	55.13±8.80	155.59

The mean diurnal rate for every two hours was calculated from the individual half hourly rates of observation for the days on which the sampling was conducted for six times (over day and night). The mean diurnal rate of methane emission from *Scirpus* plants grown in Bhalswa Soil was highest in the morning that coincided with the time of the peak rate of emission (Fig.17).

The mean diurnal rate of methane emission from *Scirpus* plants grown in Sandy soil was highest in between 4:00 and 6:00 hr (0.76±0.27 38 mg m^{-2} hr^{-1}) and coincided with the highest individual peak rate of emission. The rate of methane emission from afternoon between 14:00 and 16:00 hr (0.609±0.26 38 mg m^{-2} hr^{-1}) followed next. In the morning from 7:00-9:00 hr the rate of emission was 0.59±0.18 38 mg m^{-2} hr^{-1}. The rate of methane emission was least at night between 20:00 and 22:00 hr (0.39±0.19 mg m^{-2} hr^{-1}).

The mean diurnal rate of methane emission from *Scirpus* plants grown in garden soil was highest in between 14:00 and 16:00 hr (18.51±3.57 mg m^{-2} hr^{-1}) and did not coincide with the peak rate of emission between 5:00 and 5:30 hr. The mean rate of emission was also very high at night 18.41±5.11 mg m^{-2} hr^{-1} compared to the rate at other time of the day. It was least in the morning 7:00-9:00 hr (14.59±1.69 mg m^{-2} hr^{1}).

Mean Diurnal Methane Emission

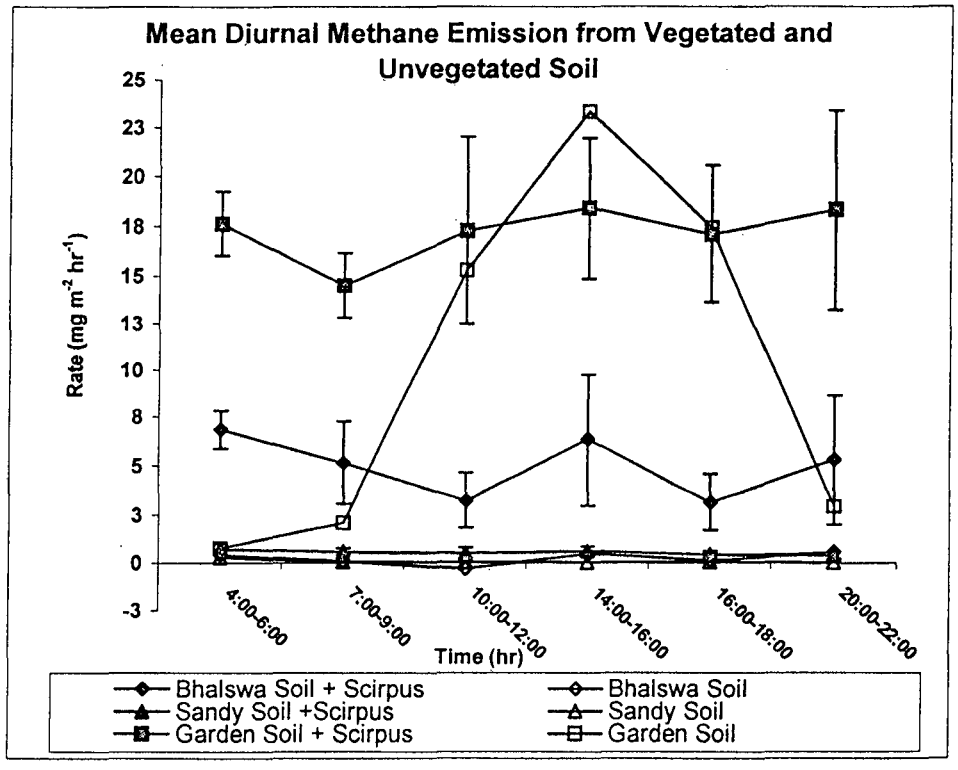


Fig.17. Mean diurnal rate of methane emission from vegetated and unvegetated soil.

The comparison (Fig.18 and 19) of the mean daily rate of methane emission from vegetated soil and unvegetated soil over four months showed that the rate of emission was higher from former than later. In May, the rate of methane emission from vegetated soil was 7.15 and 4.19 times more than in bare Bhalswa and Sandy soil respectively. In June, the rate of emission from vegetated soil was higher by 15, 2.22, 4.198 times compared to unvegetated soil in Bhalswa, Sandy and Garden soil, respectively. In October, the vegetated Bhalswa and sandy soil emitted methane at a rate about 4.15 and 2.7 times more than the unvegetated soil. As an exception in October, the overall rate of methane emission was low and the unvegetated Garden soil emitted methane at a rate of 2.45 times compared to the vegetated soil. In general, the difference in the rates between the vegetated and unvegetated soil increased from May to June, reduced in July and again increased in October except for the Garden soil

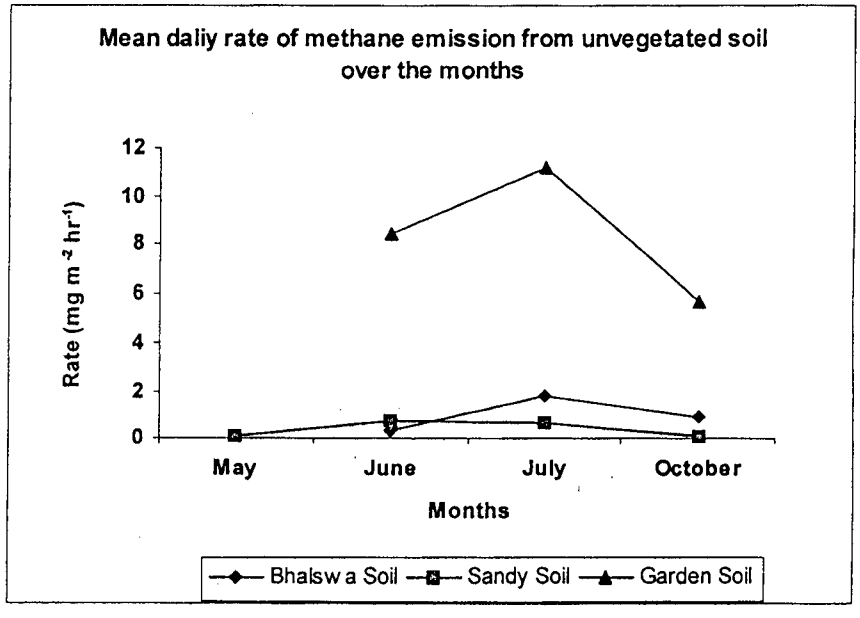


Fig.18. Comparison of the mean daily rate of methane emission from bare soil for over four months.

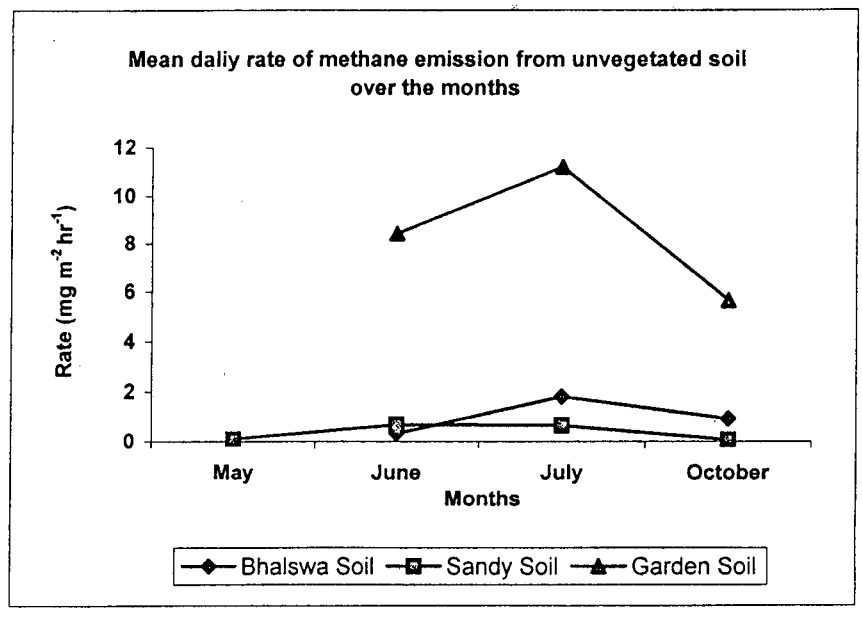


Fig.19. Comparison of the mean daily rate of methane emission from vegetated soil for over four months.

Total Organic Carbon and Total Methane Emission

A positive and significant correlation was observed (Fig.20) between the organic carbon (%) and total methane emission (mg m^{-2}). The coefficient of correlation between organic carbon and total methane emission from *Scirpus* plants grown in Bhalswa, Sandy and Garden soil was 0.94, 0.73 and 0.65, respectively.

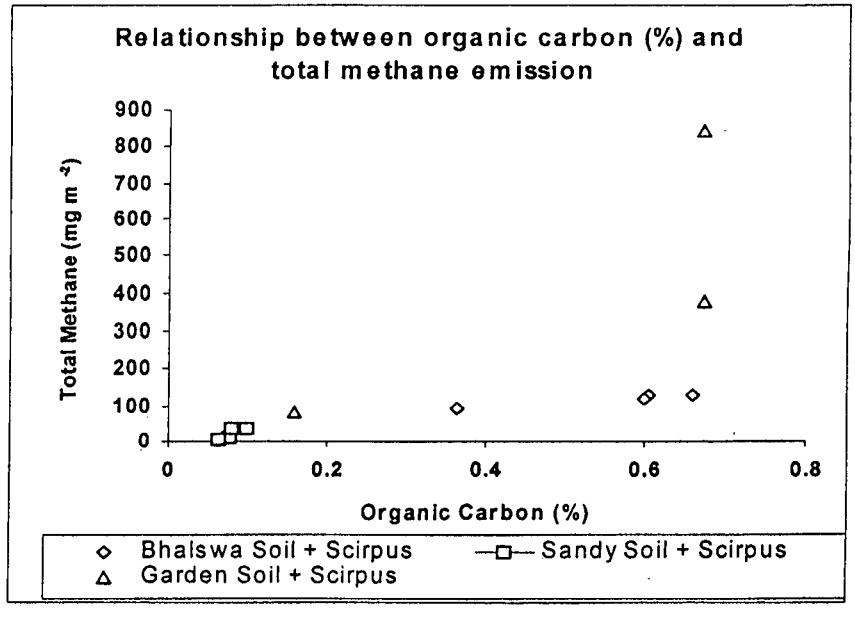


Fig.20. Relationship between total organic carbon (%) and total methane emission (mg m^{-2})

Plant Biomass

Plants grown in different soils differed in its biomass (dry weight) (Fig.No.21). The biomass of the *Scirpus* plants grown in Bhalswa Soil increased from 2077.21±140.68 g m^{-2} (for 95±7 shoots) in May, 2777.10±319.55 (for 96±12 shoots) in June to 2817.54±143.40 g m^{-2} (for 113±4 shoots) in July and decreased to 1036.25 g m^{-2} (for 61 shoots) in October.

The biomass of the *Scirpus* plants grown in Sandy soil increased from 1934.64±33.52 gm^{-2} (for 105±8 shoots) in May, to 2287.0±183.69 gm^{-2} (for 98±6 shoots) in June and reduced to 1909.68±194.35 gm^{-2} (for 78±8 shoots) in July and 1036±137.88 gm^{-2} (47±4) October.

The *Scirpus* plants growing in the Garden soil showed higher biomass content compared to the other two types of soil. The dry weight of the plants reduced from 3734.57±350.37 g m^{-2} (for 140±9 shoots) in June to 3658±393.94 g m^{-2} (for 122±15 shoots) in July and further to1593±93.91 g m^{-2} (for 61±3 shoots) in October.

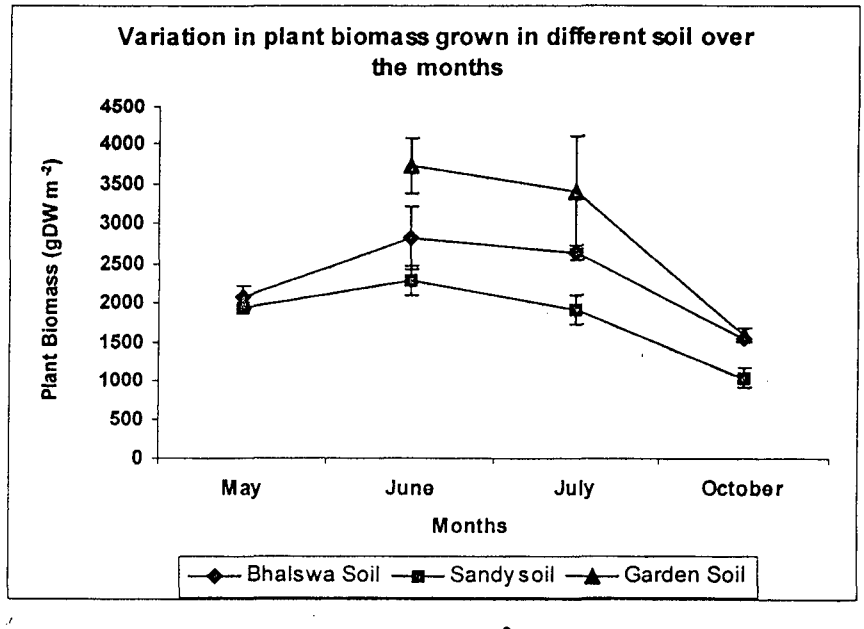
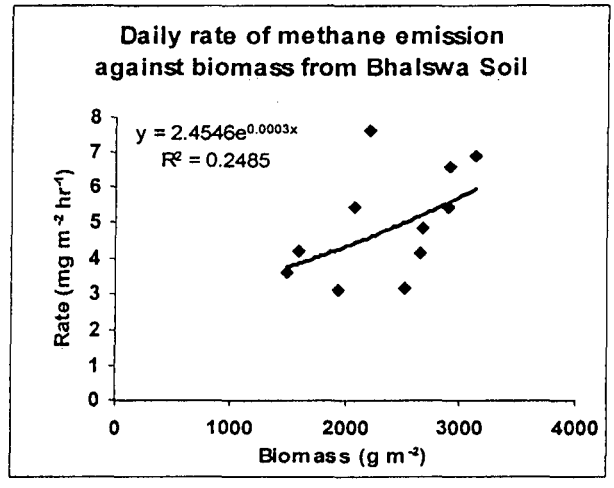
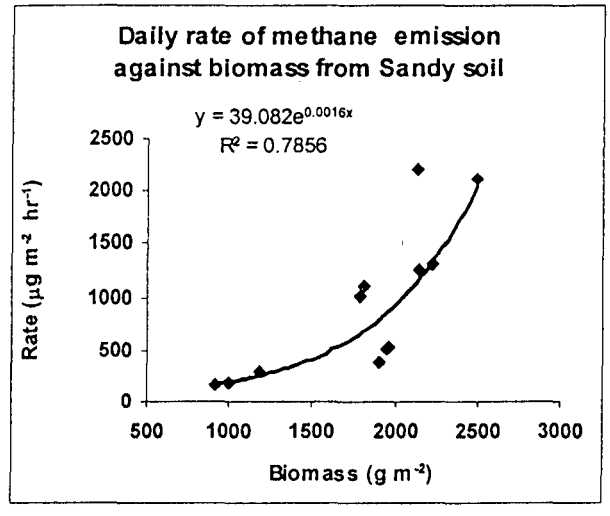


Fig.21. The variation in the plant biomass (gDW m^{-2}) grown in Bhalswa, Sandy and Garden soil over the months.

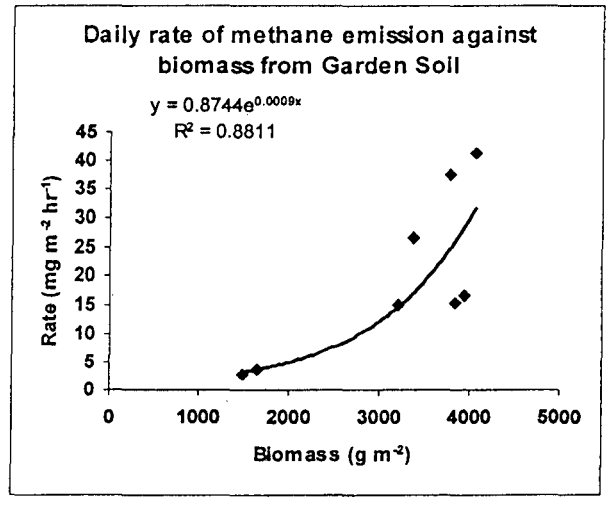
Plant Biomass and Methane Emission



a)



b)



c)

Fig. 22. Variation in daily rate of methane emission (mg $m^{-2}hr^{-1}$) against plant biomass (g m^{-2}) from a) Bhalswa, b) Sandy and c) Garden soil.

Exponential trendline (order 2) was best suited to bring forth the correlation between the daily rate of methane emission and plant biomass. The coefficient of correlation between the daily rate of methane emission and plant biomass was positive in all types of soil but it was very weak in Bhalswa soil. Only 7% (r^2=0.23) variation in the increase in methane emission could be explained with the increase in biomass (Fig.22a). In Sandy soil 60.52 % (r^2=0.79) (Fig.22b) of variation in the increase in methane emission was explained by the increase in biomass whereas 74.32% (r^2=0.88) variation in the increase in methane emission with the increase in biomass of plants in Garden soil (Fig.22c) could be explained.

Influence of water level on methane emission

Scirpus plants grown in garden soils were subjected to two different water levels of 15 cm (2 tanks) and 30 cm (2 tanks) after a prolonged period of dryness (~15 days) (Fig. 23). The other two tanks with the plants had water level 1-2cm below surface. The rate of emission of methane was investigated thereafter at the end of June and first week of July.

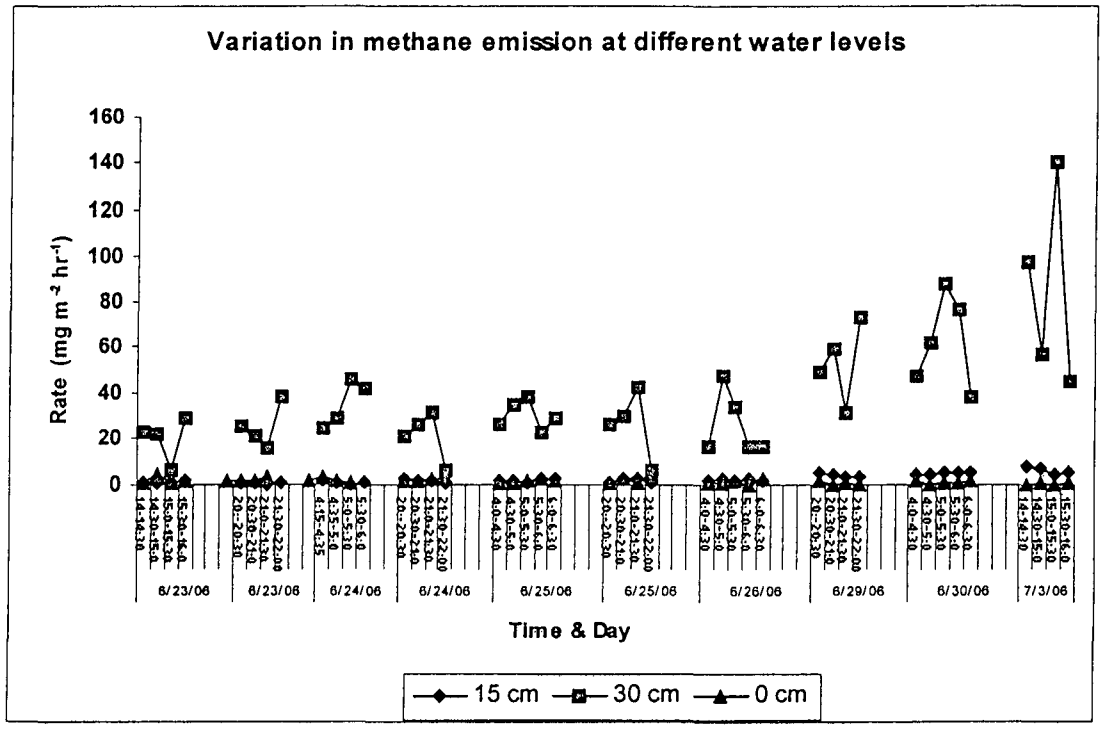


Fig.23. Variation in the rate of methane emission (mg m^{-2} hr^{-1}) in different water levels

The rate of emission differed with water level (Fig.23.). The concentration of methane in the chamber that enclosed plants with water level below soil surface did not change

with time. The concentration was close to the atmospheric methane concentration and the rate did not change in twelve days (end of June and first week of July) in these tanks. The rate of emission was initially low in plants that had water at a level of 15cm above the soil surface. At the end of twelve days the average rate of emission increased from 1.05±0.18 mg $m^{-2} hr^{-1}$ to 5.97±1.66 mg $m^{-2} hr^{-1}$. The rate of emission was high in plants with water level at 30cm above the soil surface. The average rate of emission ranged from 19.8±8.9 mg $m^{-2} hr^{-1}$ on the first day of sampling to 84.70± 39.91 mg $m^{-2} hr^{-1}$ on the twelfth day of sampling.

Oxidation of methane

The highest rate of methane oxidation was observed from primary roots followed by roots and rhizomes, shoot parts and the rhizospheric soil (Table 9).

Table 9. Oxidation of methane by parts of plants

Parts of Plants	**Oxidation of Methane ($\mu g\ g^{-1}$ DW hr^{-1})**
Shoots	14.66±1.69
Roots and Rhizomes	31.61±11.05
Primary Roots	91.48±8.05
Rhizospheric Soil	3.77±0.95

The highest rate of methane oxidation was observed from primary roots followed by roots and rhizomes, shoot parts and the rhizospheric soil.

Methane Production from a Natural Wetland containing Peat Soil

Characteristic of the Peat collected from Lake Khajjiar in 2004

The average pH of the peat prior to incubation was 6±2 and it did not differ across the three different layers (0-10, 10-15, 15-20 cm) of the soil core at all the sites. The organic matter content of 15-20cm soil layer was highest followed by 10-15 and 0-10 cm layer at Site A and B at Lake Khajjiar (Table 10).

Table.10. Organic matter content (%) of soil from the two sites at Lake Khajjiar.

Sites	0-10cm Soil layer	10-15cm Soil layer	15-20cm Soil layer
A	42.95	44.51	51.46
B	37	43	56

The organic matter content of 15-20cm soil layer was highest followed by 10-15 and 0-10 cm layer at Site A and B at Lake Khajjiar (Table 10).

Methane and Carbon dioxide production from soils at Site-C

A preliminary experiment was conducted with the three different soil layers at Site-C to determine the production and oxidation capacity of the peat soil at Lake Khajjiar. The experiment was terminated after 15 days. The rate of methane and carbon dioxide production varied across different layers of soil at 25°C, at Site C, Lake Khajjiar (Table 11). The rate of methane production via hydrogenotrophic methanogenesis (soil slurries incubated with methyl fluouride) was less than the total methanogenesis. Respiratoy Index (RI) was higher in the 10-15cm and 15-20cm layer compared to the 0-10cm layer. Methane (supplemented externally) was consumed by the three soil layers. The 0-10cm soil layer consumed the most methane followed by the 10-15cm soil layer and 15-20cm soil layer at Site-C. The preliminary experiment showed that methane was produced and also oxidized by the soil at Lake Khajjiar, but the major work with the soil collected from Lake Khajjiar in 2004, focussed on methane production.

Table 11. Rate of methane and carbon dioxide production from soil of 0-10, 10-15, 15-20 cm layers at Site-C

Site C	0-10cm Layer		10-15 cm Layer		15-20 cm Layer	
	Control Mean ± SD	CH_3F inhibited samples Mean ± SD	Control Mean ±SD	CH_3F inhibited samples Mean ± SD	Control Mean ± SD	CH_3F inhibited samples Mean ± SD
CH_4 production ($\mu mol\ g^{-1}DW\ day^{-1}$)	3.45±0.69	0.9±0.45	1.60±0.47	0.8±0.69	2.28±0.61	1.0±0.173
CO_2 production ($\mu mol\ g^{-1}DW\ day^{-1}$)	3.78±1.48	n.a.	2.64±0.35	n.a.	3.65±0.35	n.a.
RI $\{(CO_2/CH_4+CO_2)\}$	0.52	n.a.	0.62	n.a.	0.62	n.a.
CH_4 Consumption ($\mu mol\ g^{-1}DW\ day^{-1}$)	13.46±3.51	n.a.	7.76±3.05	n.a.	11.30±2.47	n.a.

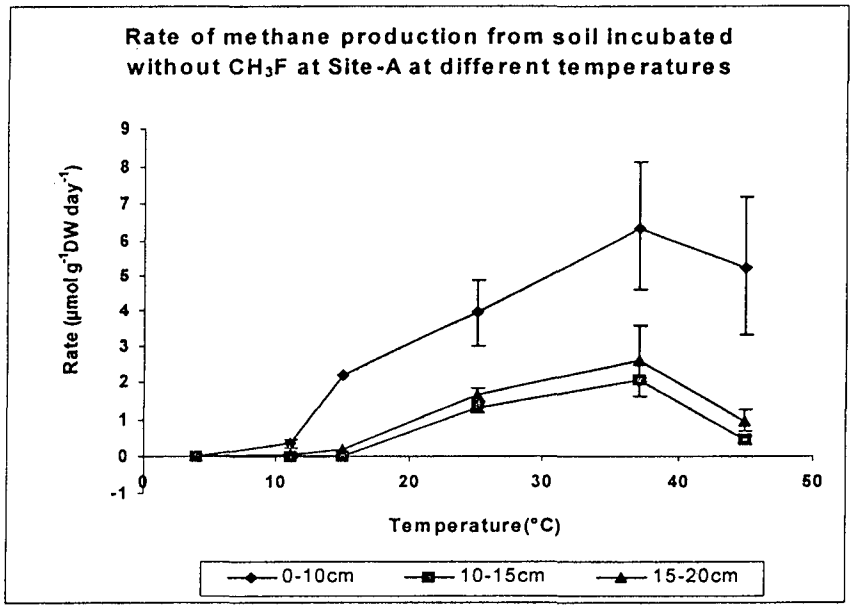
Rate of methane, carbon-dioxide and hydrogen production at different temperatures from Site-A and B.

Slurries were prepared with soil of different layers at Site A and Site B and incubated with and without methyl fluoride at different temperatures (4 to 45°C). They differed in their rate of methane, hydrogen and carbon dioxide production.

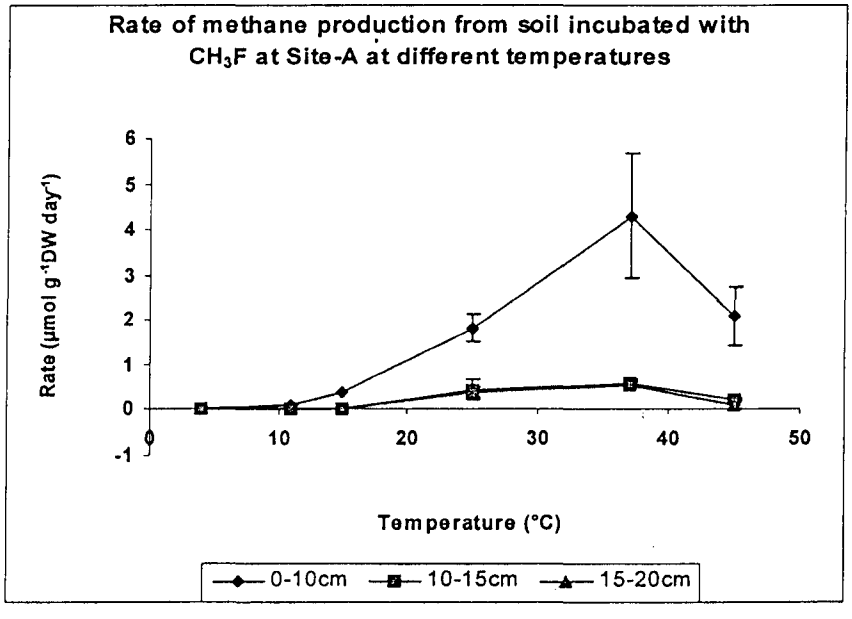
Site-A

The soil from different depths at Site A was incubated for 30 days. The rate of methane production increased from 4° C until 37°C and then decreased at 45°C in the soil across all layers; 0-10cm, 10-15cm and 15-20cm (Fig.24a). The rate of methane production was highest at the 0-10cm soil layer, followed by the 10-15cm and 15-20cm soil layer.

The addition of methyl fluoride inhibited methane production from acetoclastic methanogenesis. The methane produced in the soils with methyl fluoride is from hydrogenotrophic methanogenesis. The rate of methane production from soil incubated with methyl fluoride was less compared to the soil incubated without methyl fluoride (Fig.24b).

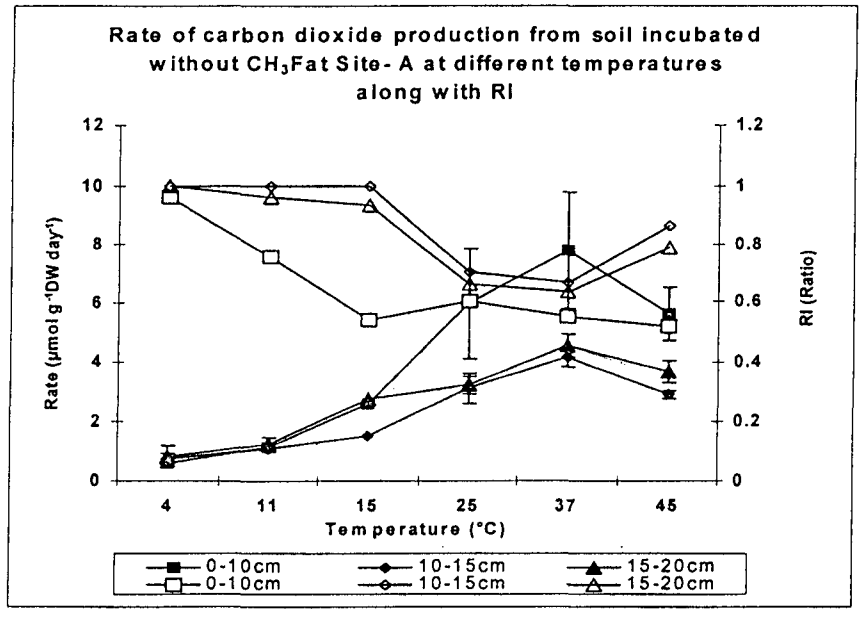


a)

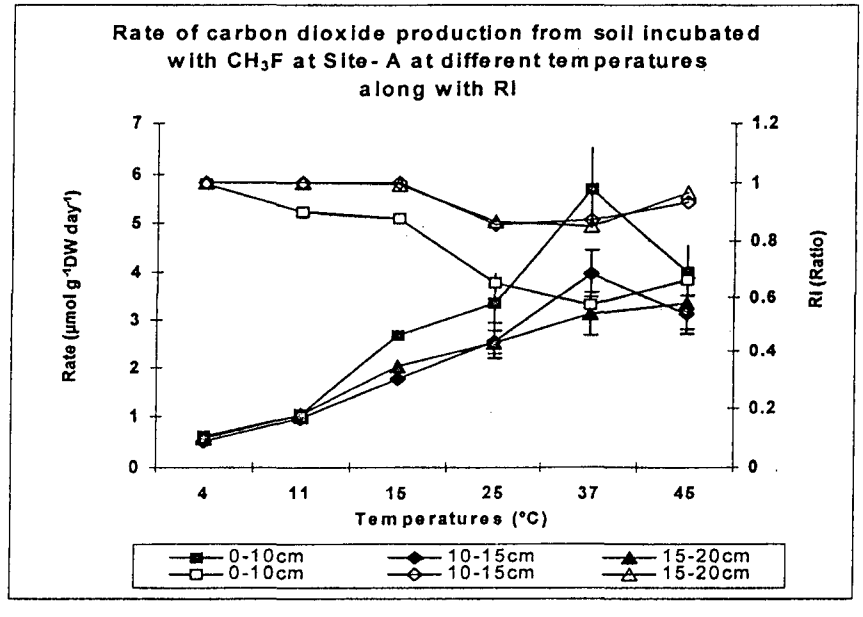


b)

Fig.24 Variation in the rate of methane production (µmol g^{-1}DW day^{-1}) from soil of three different depths a) without and b) with CH_3F at Site-A with the rise in temperature.

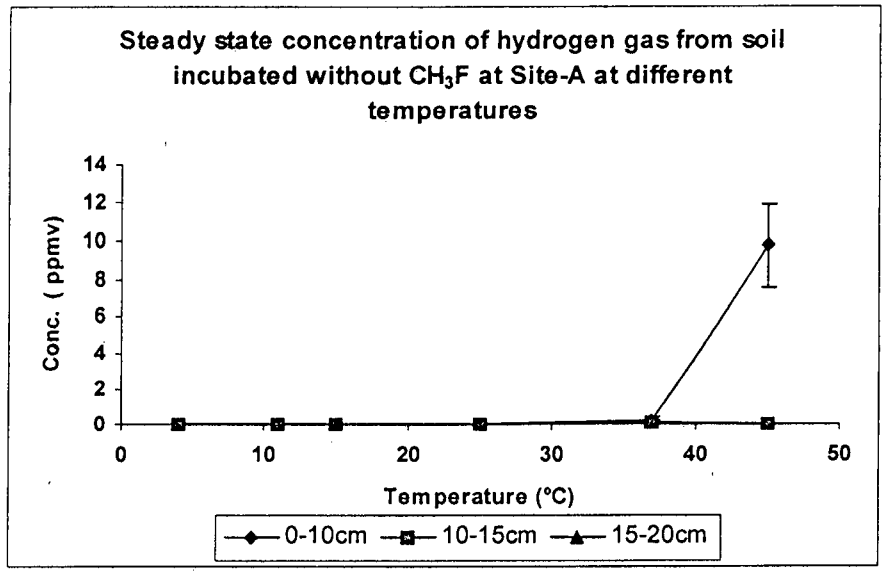


a)

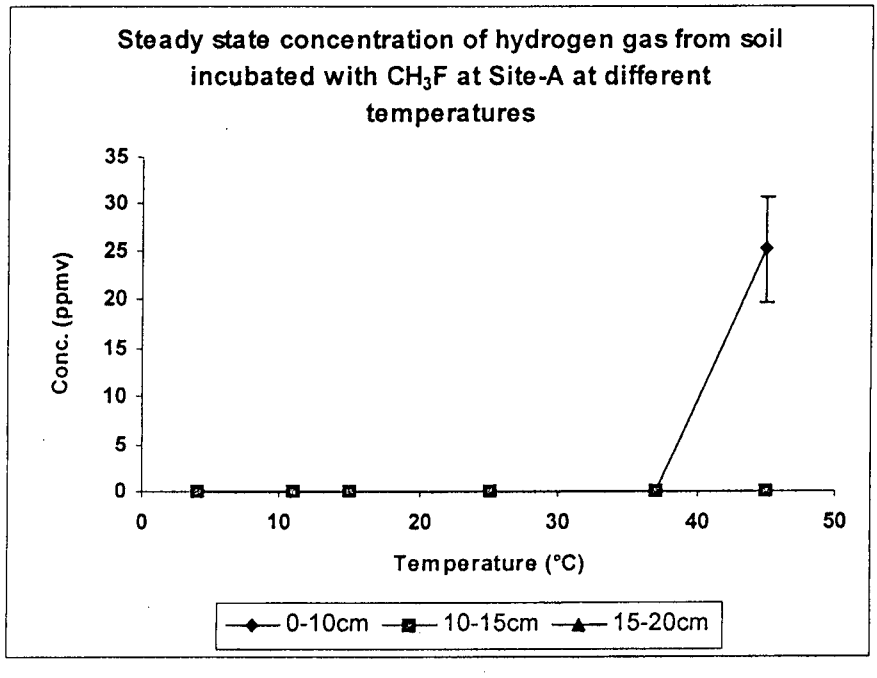


b)

Fig.25. The variation in the rate of carbon dioxide production (µmol g^{-1} DW day^{-1}) from soil of three different depths a) without and b) with CH_3F at Site-A with the rise of temperature. Respiratory Index is represented in open symbols.



a)



b)

Fig.26. Steady state concentration of hydrogen gas (ppmv) from soil of three different depths a) without and b) with CH_3F at different temperatures at Site-A

The rate of carbon dioxide production was higher than the rate of methane production from soil incubated without and with methyl fluoride at different layers. The rate of carbon dioxide also increased until 37°C and then decreased at 45°C in soils incubated with and without methyl fluoride (Fig 25a, b).

The respiratory index (RI=CO2/CO_2+CH_4) was very high (~ 1) at 4°C and decreased (~ 0.5) with the increase in temperature in soils incubated with and without methyl fluoride from 0-10cm layer at Site A (Fig 25a, b).

The steady state concentration of hydrogen gas was low and observed only at 37°C and at 45°C from the 0-10cm soil layer, at 37°C from the 10-15 cm soil layer and from the 15-20 cm soil layer (Fig.26a). In the inhibited soil, the steady state concentration was 25.27±5.54 ppmv at 45°C from the 0-10cm layer (Fig.26b).

Site B

The soil from three different layers 0-10 cm, 10-15cm, 15-20 cm at Site B were incubated for 45 days (Fig.27a). The rate of methane production from 0-10cm soil layer at Site-B was higher than 10-15cm and 15-20cm. The rate of methane production was negligible at lower temperatures and increased with the rise in temperature until 37°C and decreased thereafter from soils across different depths.

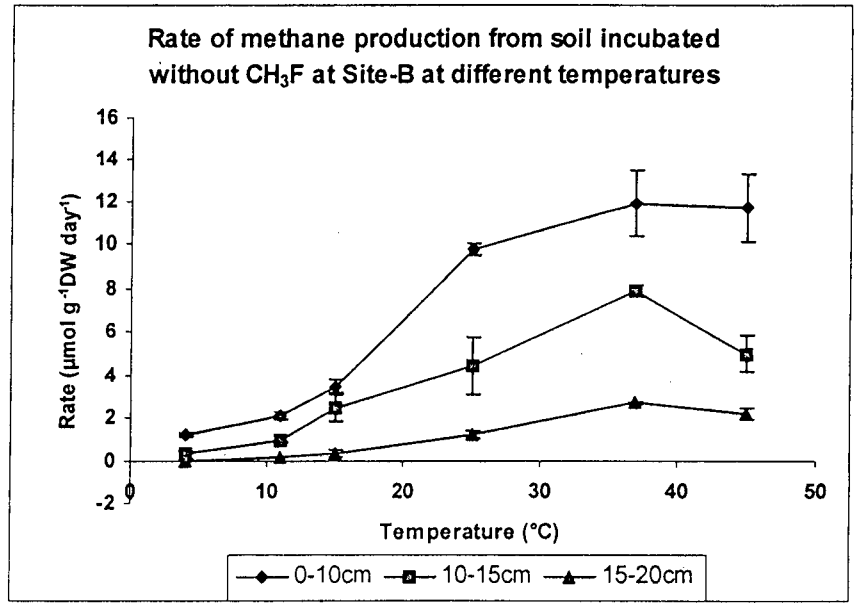
The rate of methane production from the soils incubated with methyl fluoride (Fig.27b) was lower than the soils incubated without methyl fluoride.

The rate of carbon dioxide production was higher (Fig.28a, b) than the rate of methane production from the soil incubated without and with methyl fluoride at different layers. The rate of carbon-dioxide production increased until 37°C and then reduced at 45°C from the soil of different depth at Lake Khajjiar.

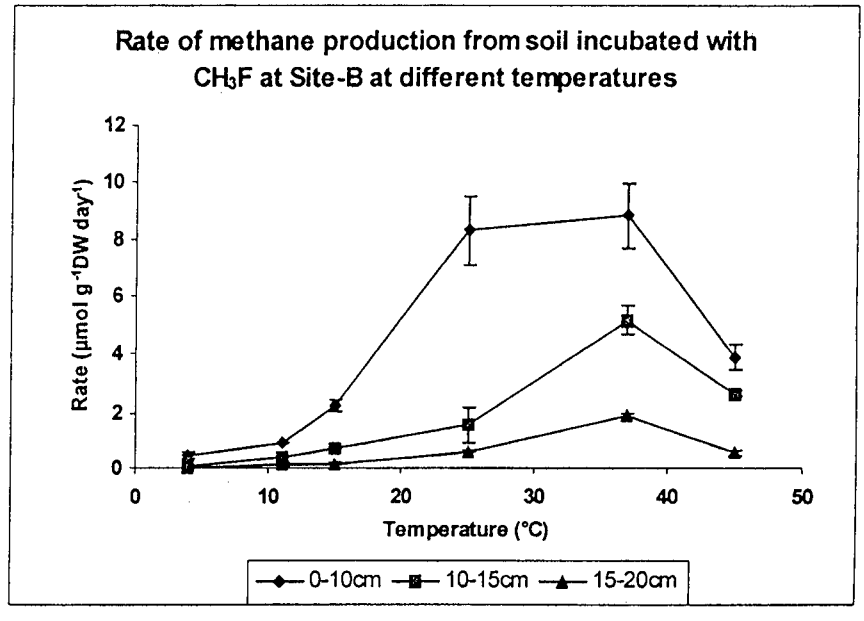
The Respiratory Index (Fig.28a) was around 0.5 in soil at 0-10cm layer. The RI in the other two soil depths was higher than 0-10cm layer in the soils incubated without methyl fluoride.

The Respiratory Index was (Fig.28b) higher in soils incubated with methyl fluoride than soils without incubated with methyl fluoride at Site B.

The steady state concentration of hydrogen gas increased with temperature in the soil incubated with methyl fluoride and without methyl fluoride but the fluctuation was more in the soils without methyl fluoride (Fig.29a, b).

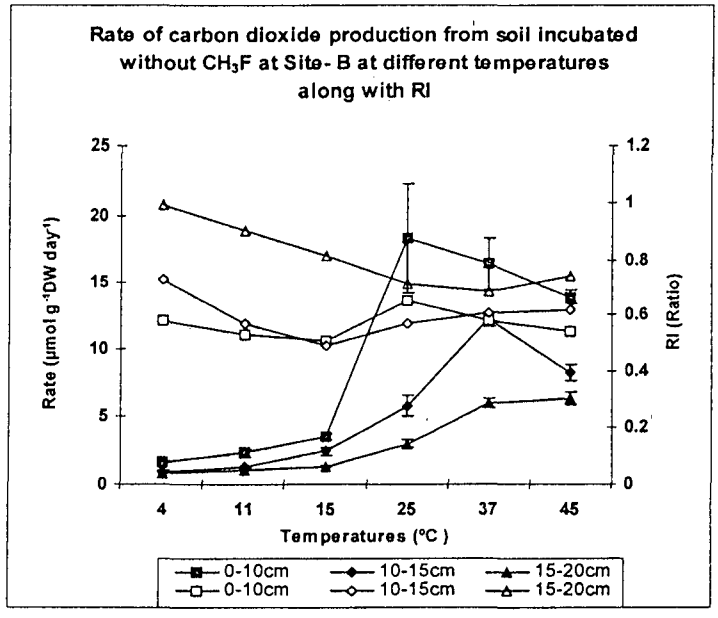


a)

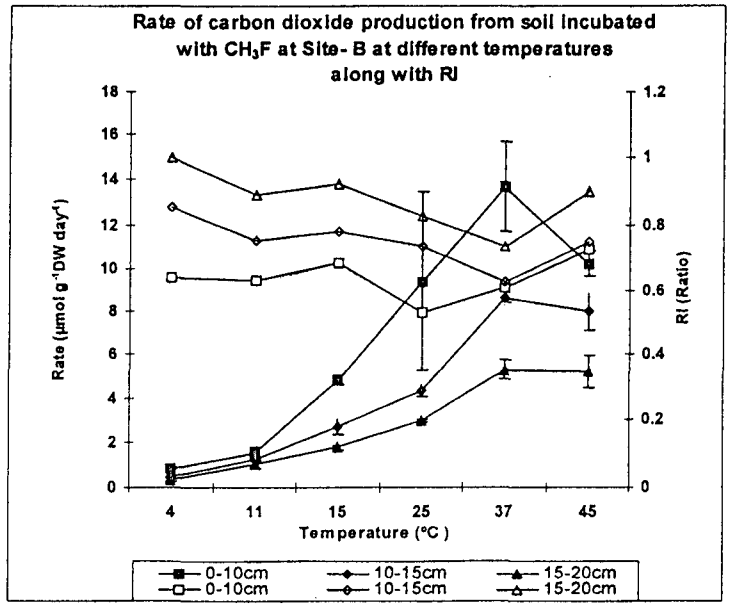


b)

Fig. 27. The variation in the rate of methane production (µmol g^{-1} DW day^{-1}) with the rise of temperature at three different soil layers a) without and b) with CH_3F at Site-B.

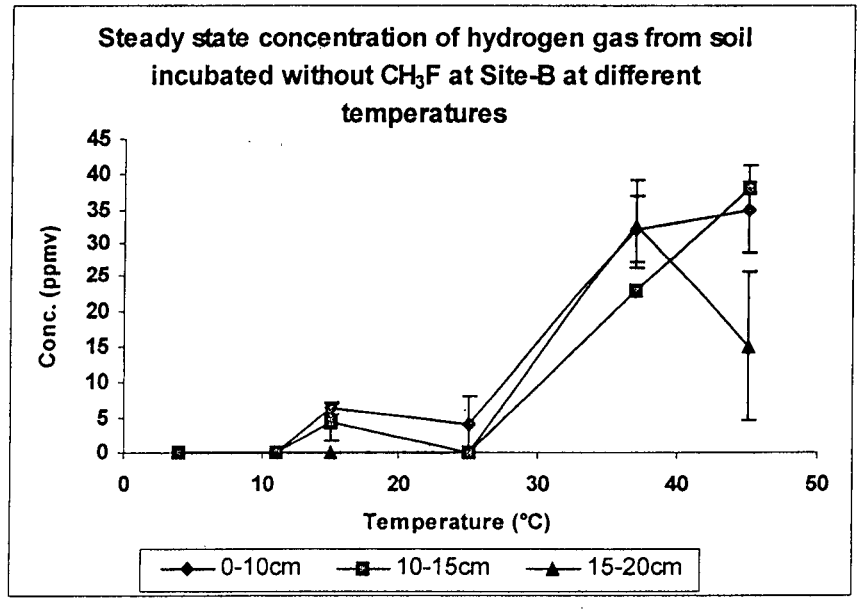


a)

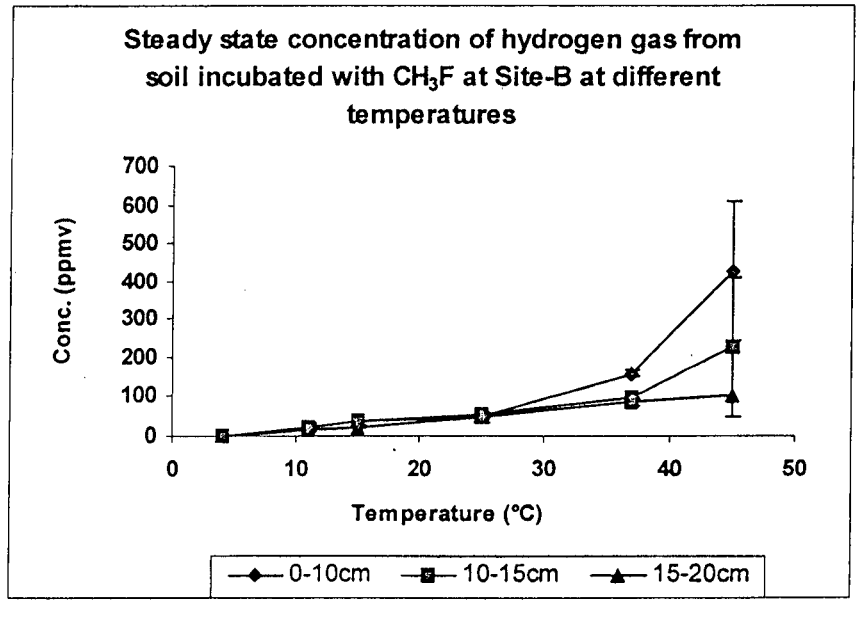


b)

Fig.28. The variation in the rate of carbon dioxide production (µmol g^{-1} DW day^{-1}) with the rise of temperature at three different soil layers a) without and b) with CH_3F at Site-B. Respiratory Index is also represented by open symbols.



a)



b)

Fig.29. Steady state concentration (ppmv) of hydrogen gas of a) without and b) with CH_3F of the three soil layers at different temperatures at Site-B

Hydrogenotrophic Methanogenesis at Site-A and B

At lower temperature acetoclastic methanogenesis was dominant and with the increase in temperature hydrogenotrophic methanogenesis took over until 37°C at both the sites (Fig.30 a and b). Polynomial trendline was best suited to bring forth the relation ship between hydrogenotrophic methanogenesis and rise in temperature at Site A (order 3; Equation; $y=-3E-05x^3+0.0018x^2-0.0261x+0.237$) and Site-B (order 3; Equation: $y=-1E-05x^3+0.0003x^2+0.0138x+0.332$) The linear rate of methane production decreased above the optimal temperature as well as the fraction of methane from H_2/CO_2. Hydrogenotrophic methanogenesis decreased with depth. This observation was found similar for both the sites. At Site A-the contribution of H_2/CO_2 to methane production increased substantially with the rise in temperature (up to 46% at 37°C in the 0-10cm layer) where as at Site B-hydrogenotrophic methanogenesis was 40% at 4°C and increased up to 70% at 37°C in the 0-10cm layer.

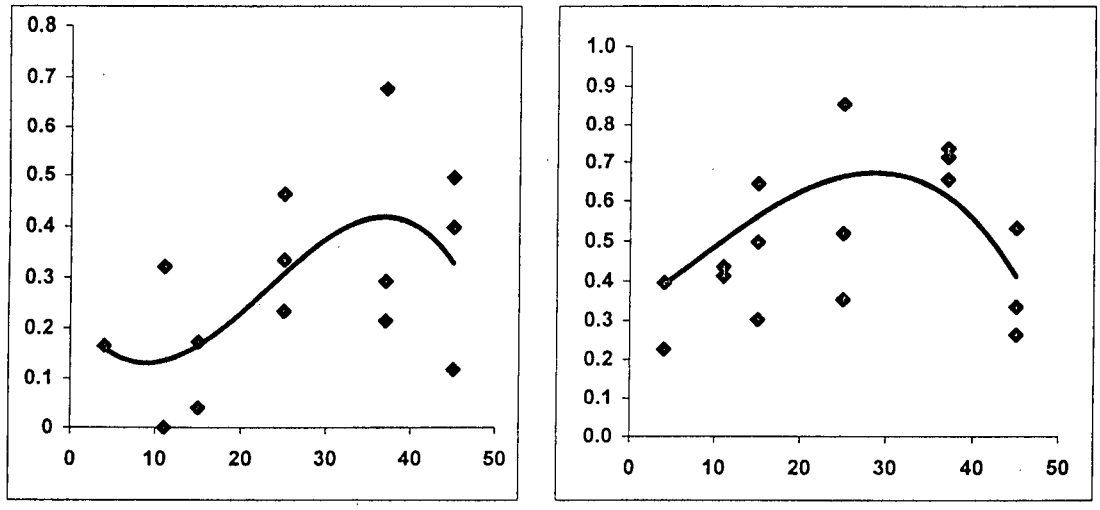


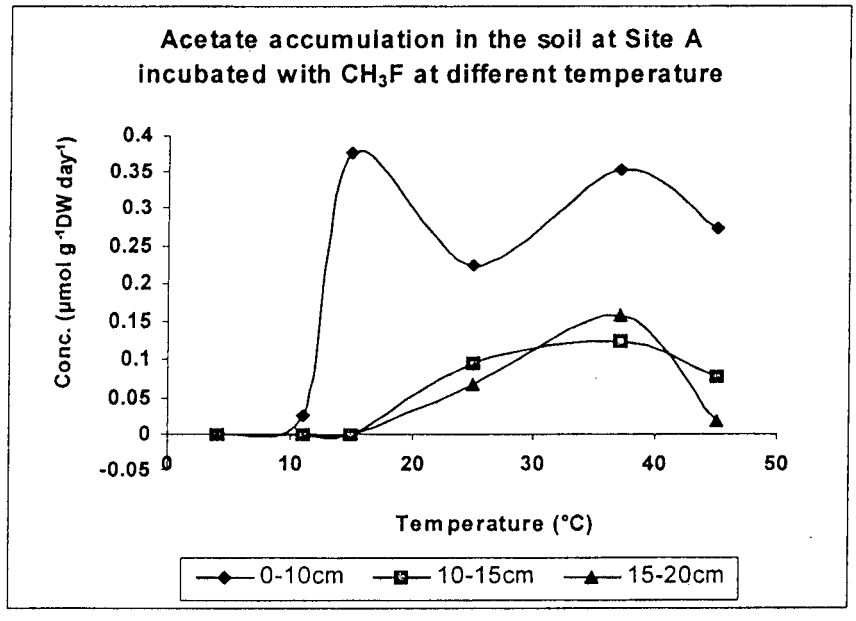
Fig.30 Contribution of methane produced by hydrogenotrophic methanogenesis with the rise of temperature at a) Site A and b) Site B

Fatty Acid Analysis

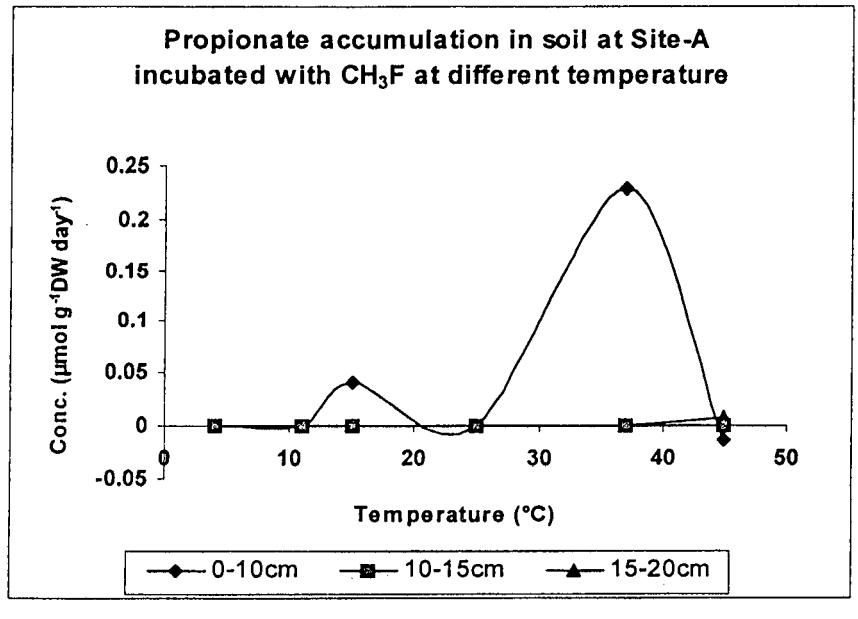
At Site-B, propionate (Fig.32b) was not observed in soils incubated over the wide range of temperature except at few temperatures (0.057 μmol g^{-1} DW day^{-1} in the 10-15 cm layer at 25°C, 0.001 μmol g^{-1} DW day^{-1} in the 0-10cm layer at 37°C, 0.237 μmol gDW^{-1} day^{-1} in the 0-10cm layer at 45°C, 0.367 μmol g^{-1} DW day^{-1}in the 10-15 cm layer at 45°C and 0.073 μmol g^{-1} DW day^{-1} in the 15-20 cm layer at 45 °C). The

transient accumulation of propionate was highest at 45°C in the soils incubated with methyl fluoride from 0-10cm and 10-15 cm layer at Site B.

Butyrate accumulated in two samples (data not shown) incubated with methyl fluoride at 11°C (0.14 μM) and 45°C (0.16μM) for the 0-10cm layer only at Site B. Substances coeluting with Butyrate giving a strong signal in the UV detected but not in the RI was observed. No conclusion could be reached for this substance, which was found for nearly all the samples at Site –A and for few at Site-B.



a)

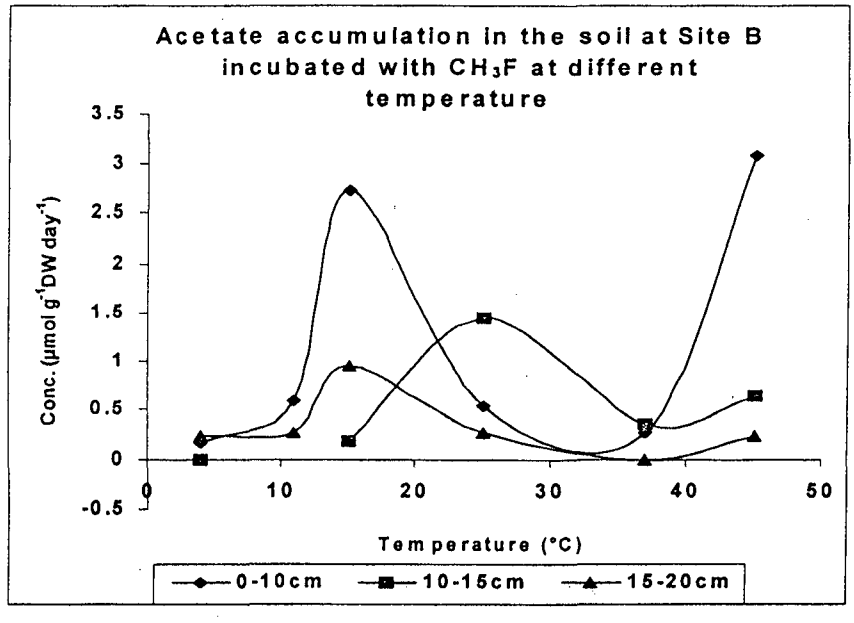


b)

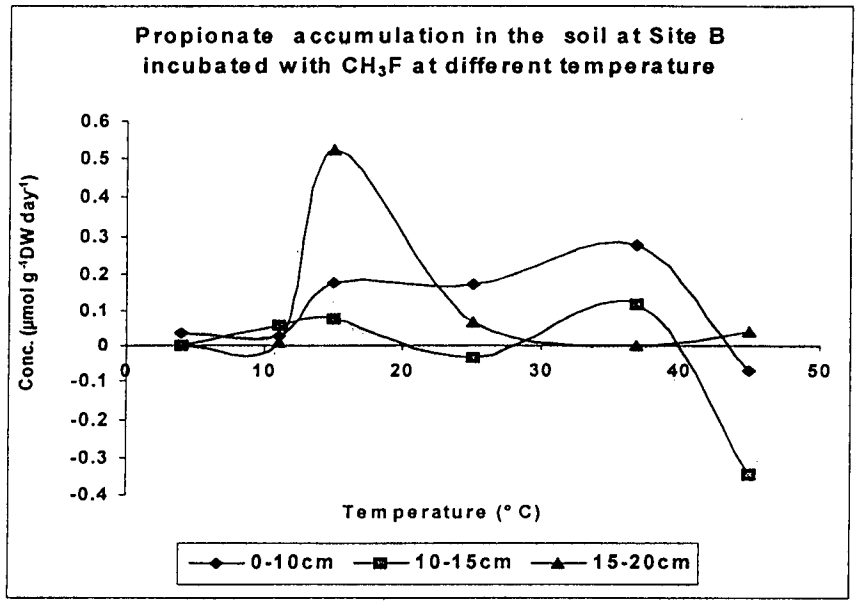
Fig. 31. Transient accumulation of a) acetate and b) propionate (μmol g^{-1} DW day^{-1})in the soil of three different depth incubated with CH_3F at Site A.

Fatty acids were nearly absent (except for acetate in the 10-15cm soil layer-0.0028μmol $gDW^{-1}day^{-1}$ and 15-20 cm soil layer-0.00383μmol gDW^{-1} day^{-1}in Site B) in the soil before incubation at different temperatures. Post incubation transient accumulation of acetate was dominant followed by propionate and butyrate. At Site-A, fatty acids were not observed in the soil incubated over the wide range of temperature except at 45°C (0.92 μmol gDW^{-1} day^{-1} in the 0-10cm layer and 0.045 μmol g^{-1} DW day^{-1}). However considerable accumulation of acetate (Fig.31a) and propionate (Fig.31b) to a much lesser extent was observed at higher temperatures, in the soils incubated with methyl fluoride at 0-10cm layer at Site-A. The transient accumulation of acetate (Fig.31a) was highest at 45°C and then at 37°C in the soils incubated with methyl fluoride at 0-10cm layer of Site A.

At Site-A, propionate (Fig.31b) was not observed in the soils incubated over the wide range of temperature except at 45°C (0.133 μmol g^{-1} DW day^{-1} in the 0-10cm layer).
At Site-B, fatty acids were not observed in soils incubated over the wide range of temperature except at 37°C and 45°C. Transient accumulation of acetate (Fig.32a) was highest at 25°C, and then at 45°C, 37°C in soils incubated with methyl fluoride from 0-10cm layer at Site B.



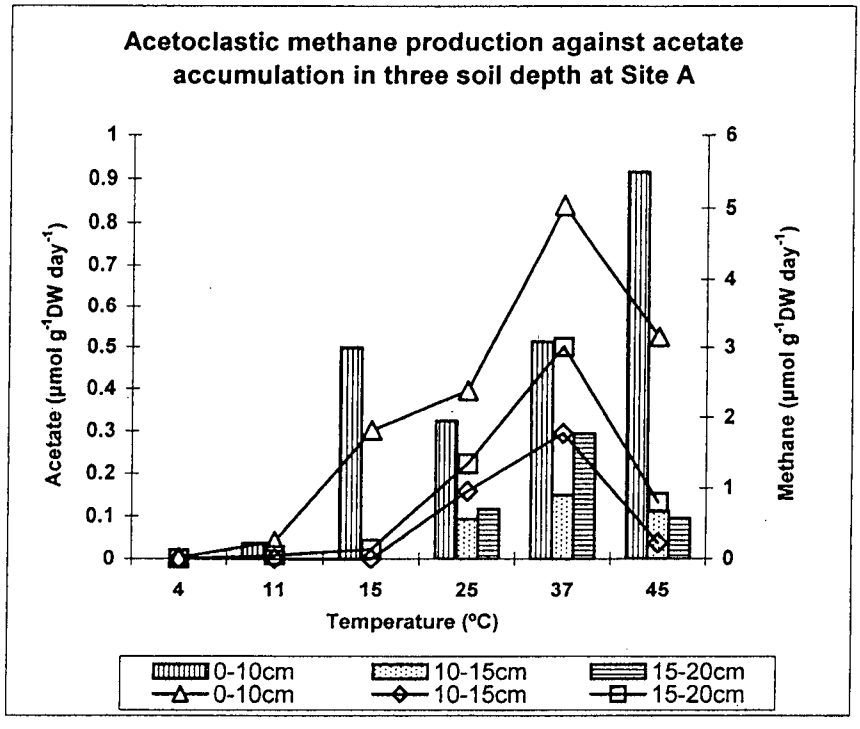
a)



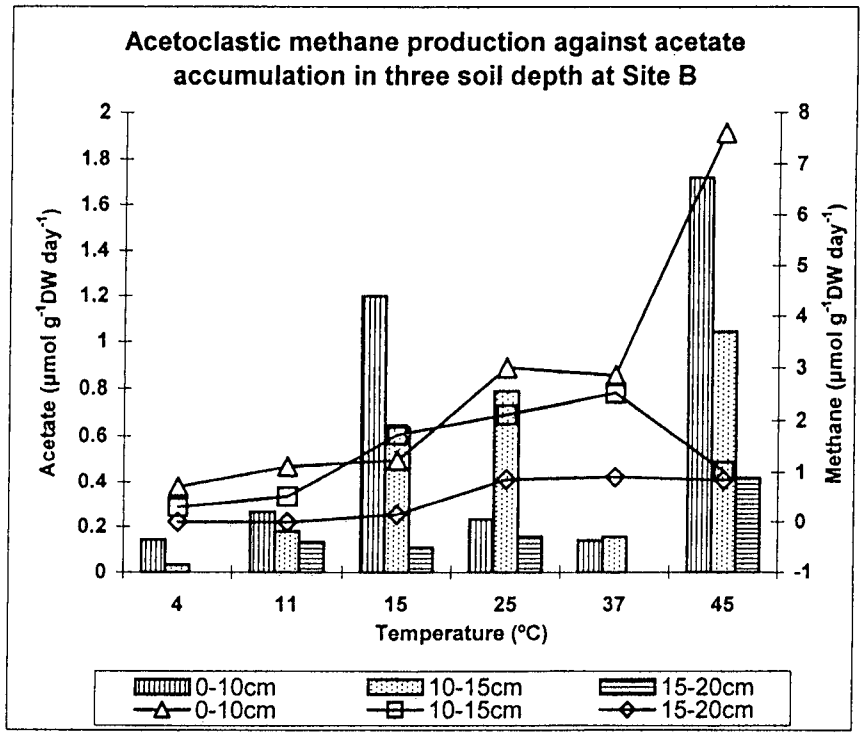
b)

Fig.32 Transient accumulation of a) acetate and b) propionate (µmol g^{-1} DW day^{-1}) in the soil from three different depths incubated with CH_3F at Site B.

Methane produced by acetoclastic pathway was calculated by substracting the methane produced by hydrogenotrophic methanogenesis (using methyl fluoride) from methane produced by total methanogenesis (without using inhibitor). Significant correlation (Fig.33a) (p= 0.05) was observed between acetate accumulation and acetoclastic methane production at the 0-10cm (r^2=0.886), 10-15 cm (r^2=0.935) and 15-20 cm (r^2=0.941) layers of soil at Site A whereas at Site B (Fig.33b) the correlation was positive but not significant at any of the soil depths.



a)



b)

Fig.33 The variation in methane produced by acetoclastic pathway and acetate accumulation in the three soil layers at a) Site A and b) Site B

Structure of the Archaeal Community

Twelve major base pair fragments were detected from the soil samples and their phylogenetic affiliation cited by different workers is given below (Table 12.). The methanogens belonging to the family *Methanomicrobiaceae* and *Methanobacteriaceae* are known to uitilize the hydrogenotrophic pathway. The methanogens belonging to *Methanosaetaceae* utilize acetoclastic pathway whereas the methanogens belonging to *Methanosarcinaceaea* basically utilize the acetoclastic pathway but can also utilize the hydrogenotrophic pathway.

Table 12. Predicted Terminal Restriction fragment (T-RF) length of archaeal 16S rRNA gene sequences from clone libraries and their phylogenetic affiliation

T-RF length (bp)	Phylogenetic Affliation	Citation
75	No Affliation found	
81	Crenarcheaota Rice Cluster VI	Chin et al., (1999)
83	*Methanomicrobiaceae*	Fey et al., (2001)
91	*Methanobacteriacaea*	Chin et al., (1999)
186	*Methanosarcinaceae* / Crenarchaeota-Rice Cluster VI	Chin et al., (1999)
246	No Affiliation found	
284	*Methanosaetaceae* / Rice Cluster V	Chin et al., (1999)
382	Crenarchaeota-Rice Cluster-III	Weber et al., (2001)
393	*Methanomicrobiales*	Glissmann et al., (2004)
494	No Affiliation found	
736	Crenarchaeota-Rice Cluster IV	Chin et al., (1999)
794	Crenarchaeota-Rice Cluster IV	Chin et al., (1999)

Site-A

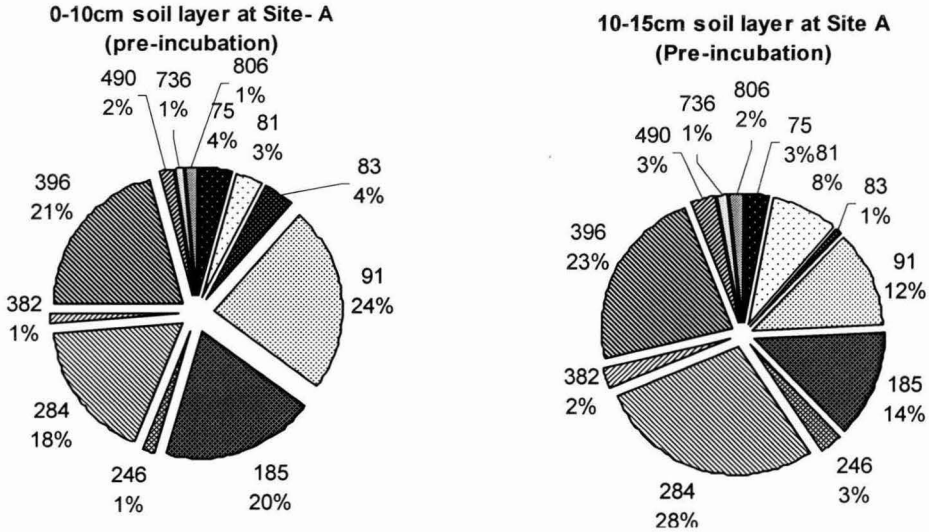
In the pre-incubated soil samples at Site-A, the relative fluorescence (%) of the dominant base pair fragments were the following

(Fig.34a), 91 bp-24%, 185 bp-20%, 284 bp-18%, 396- 21%, in the 0-10cm layer,

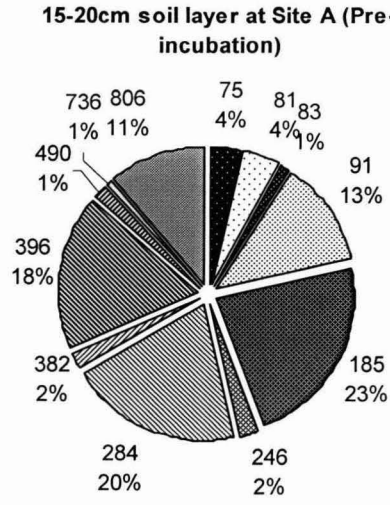
(Fig. 34b) 91 bp-25%, 185bp-14%, 284 bp-28%, 396- 23%, in the 10-15cm layer,

(Fig. 34c) 91 bp-13%, 185bp-23%, 284 bp-20%, 396-18% in the 15-20cm layer

The relative fluorescence (%) of four base pair (91, 185, 284 and 396) fragments was dominant in all the three layers of pre-incubated soil (Fig.34). Thus, for the soils incubated without and with methyl fluoride, the comparison was made with respect to the above-mentioned four base pair fragments at different temperatures.



a) b)

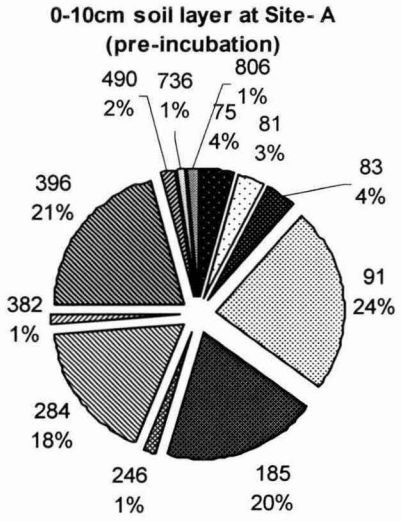


c)

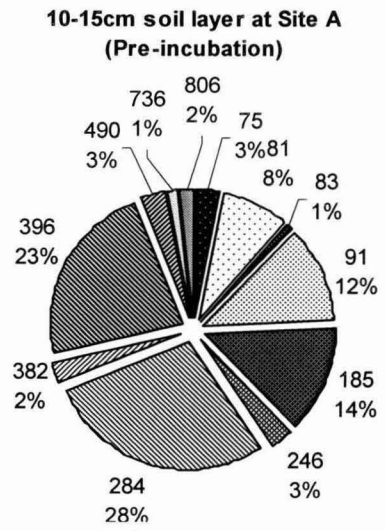
Fig.34.Variation in the relative fluorescence (%) of base pairs before incubation from a) 0-10 cm, b) 10-15 cm and c) 15-20 cm layer of soil at Site-A

In the 0-10cm layer of soil, the relative fluorescence of 91 bp was (Fig.35a) highest at 25°C and 45°C and lowest at 4°C. The relative fluorescence of 185 bp fragments (Fig. 35b) was highest at 45°C and lowest at 25°C. The relative fluorescence of 284 bp fragments (Fig.35c) was highest at 4°C and lowest at 15°C. The relative fluorescence of 396 bp fragments (Fig. 35d) was highest at 4°C and 45°C.

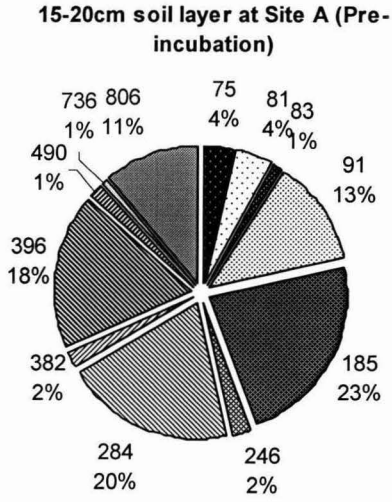
In the 0-10 cm layer of soil incubated with methyl fluoride, the relative fluorescence of 91 bp (Fig. 36a) was highest at 45°C and lowest at 11°C. The relative fluorescence



a)



b)



c)

Fig.34.Variation in the relative fluorescence (%) of base pairs before incubation from a) 0-10 cm, b) 10-15 cm and c) 15-20 cm layer of soil at Site-A

In the 0-10cm layer of soil, the relative fluorescence of 91 bp was (Fig.35a) highest at 25°C and 45°C and lowest at 4°C. The relative fluorescence of 185 bp fragments (Fig. 35b) was highest at 45°C and lowest at 25°C. The relative fluorescence of 284 bp fragments (Fig.35c) was highest at 4°C and lowest at 15°C. The relative fluorescence of 396 bp fragments (Fig. 35d) was highest at 4°C and 45°C.

In the 0-10 cm layer of soil incubated with methyl fluoride, the relative fluorescence of 91 bp (Fig. 36a) was highest at 45°C and lowest at 11°C. The relative fluorescence

of 185 bp fragments (Fig.36b) was highest at 45°C and lowest at 4 and 11°C. The relative fluorescence of 284 bp fragments (Fig.36c) was highest at 11°C and lowest at 15°C. The relative fluorescence of 396 bp fragments (Fig.36d) was highest at 45°C and lowest at 15°C.

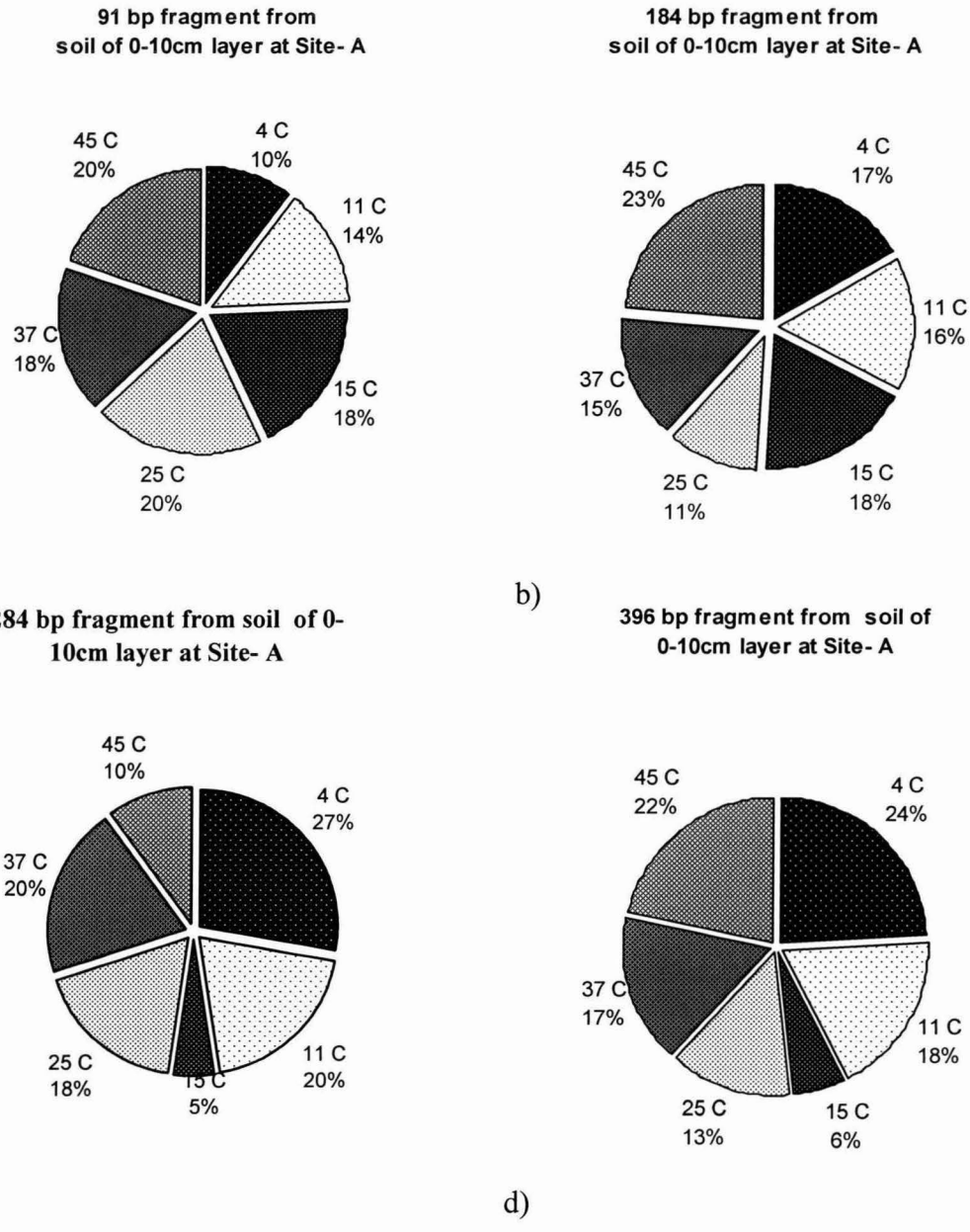


Fig.35 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil samples of 0-10 cm layer incubated at different temperatures at Site A

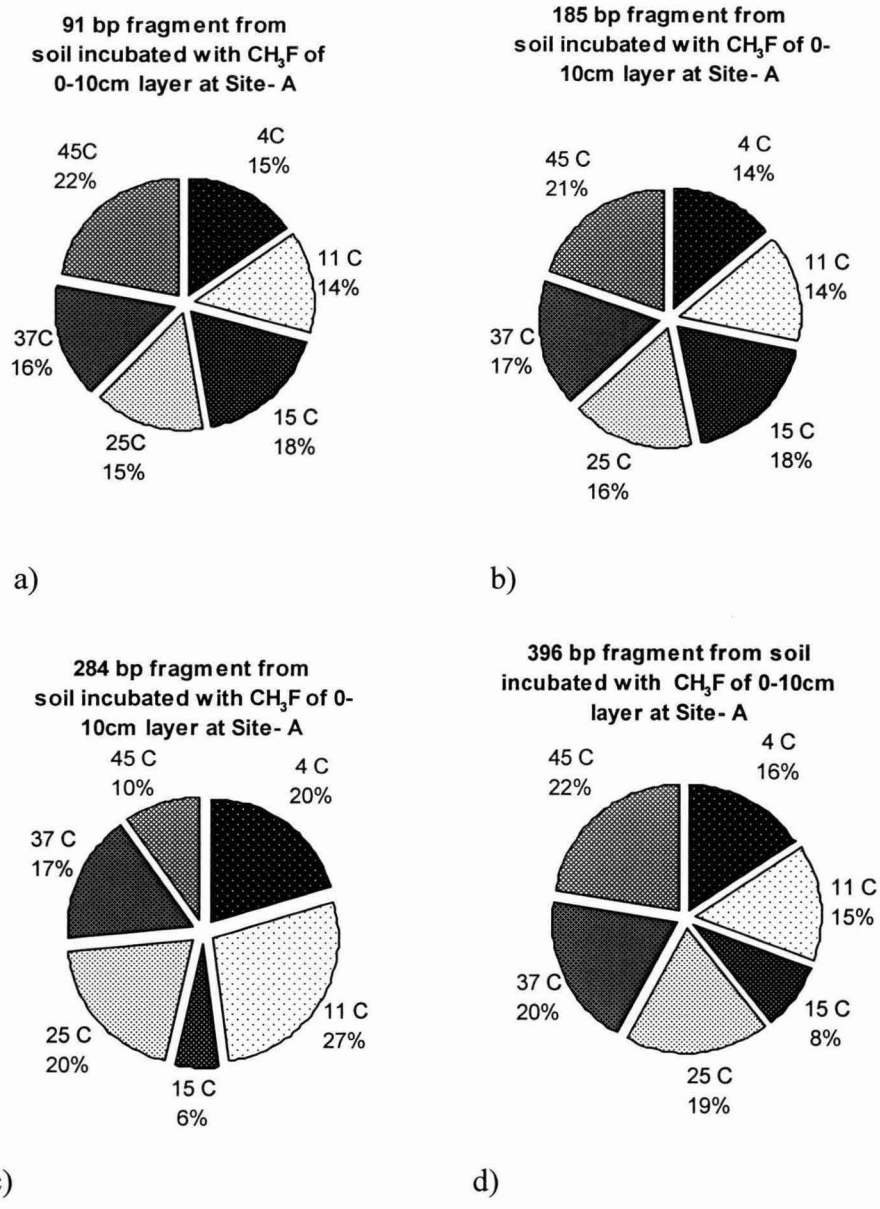


Fig.36 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 0-10cm layer incubated with methyl fluoride at different temperatures of Site A.

Site B

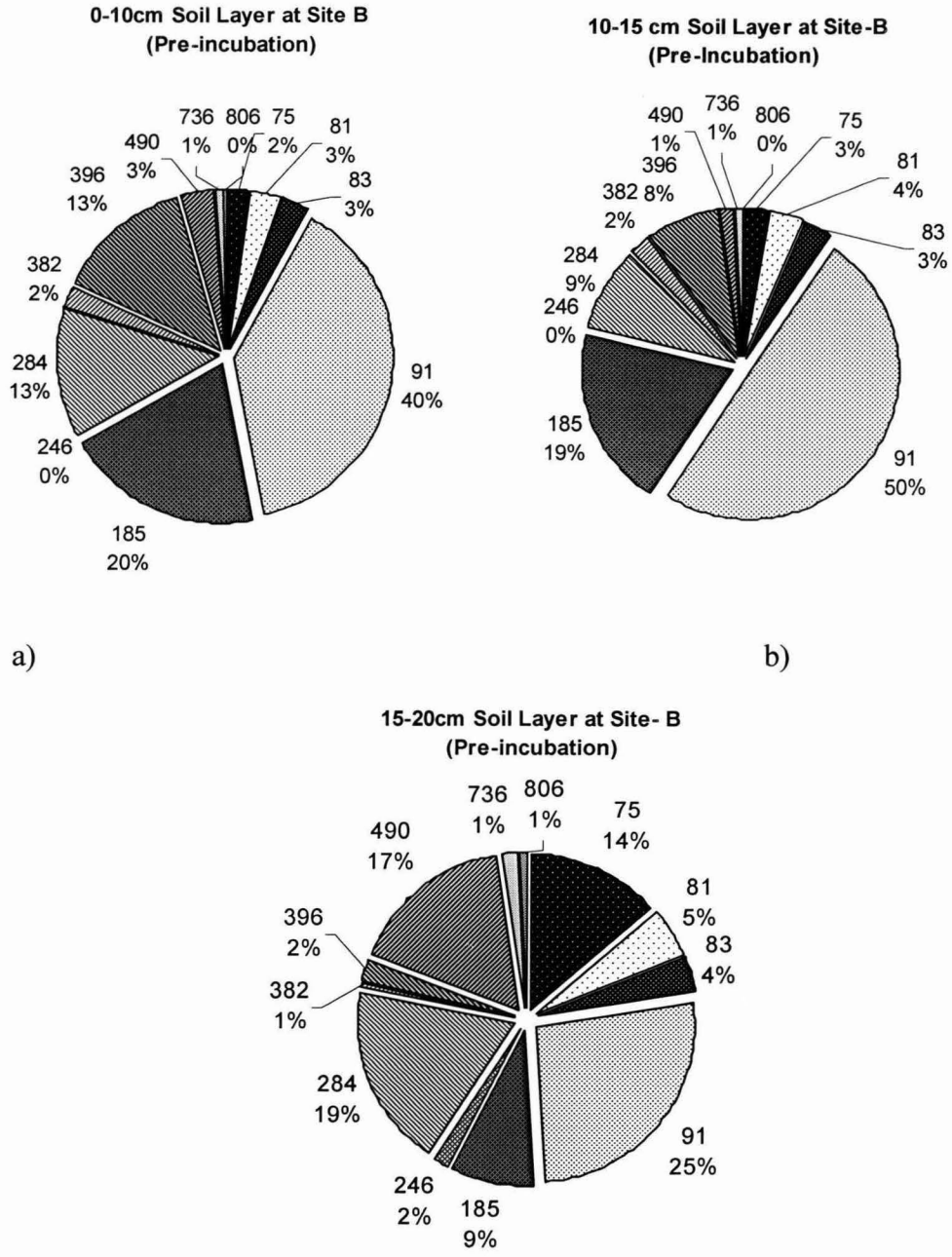
In the pre-incubated soil samples at Site-B, the relative fluorescence (%) of the dominant base pair fragments were the following (Fig.37a), 91 bp-40%, 185 bp-20%, 284 bp-13%, 396- 13%, in the 0-10cm layer,

(Fig. 37b) 91 bp-50%, 185bp-19%, 284 bp-9%, 396 bp- 8%, in the 10-15cm layer,

(Fig. 37c) 91 bp-25%, 185bp-9%, 284 bp-19%, 490 bp-17% in the 15-20cm layer

The relative fluorescence of four base pair (91, 185, 284 and 396) fragments were dominant in all three soil layers except in the 15-20cm layer where the fluorescence

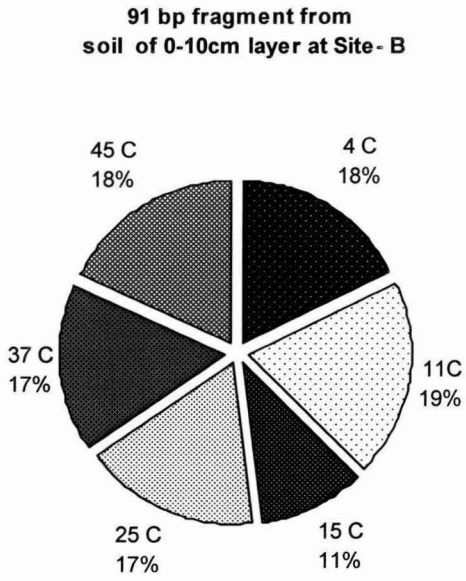
(%) of the 396 bp fragment was low and the 490 bp fragment was high. For the soil samples without and with methyl fluoride, the comparison is done in respect to the above four base pair fragments (91, 185, 284, 396) across six temperatures.



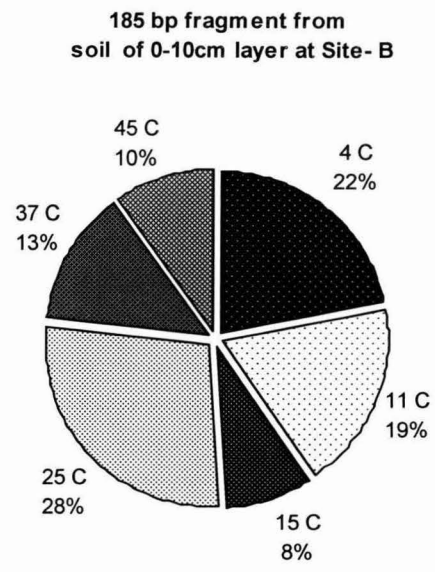
a) b)

c)

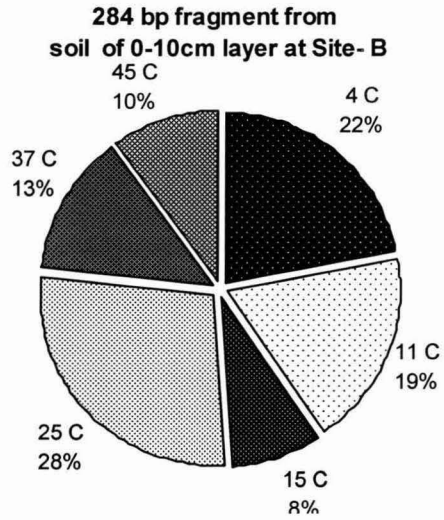
Fig. 37 Variation in the relative fluorescence (%) of base pairs before incubation from a) 0-10 cm, b) 10-15 cm and c) 15-20cm layer of soil at Site-B



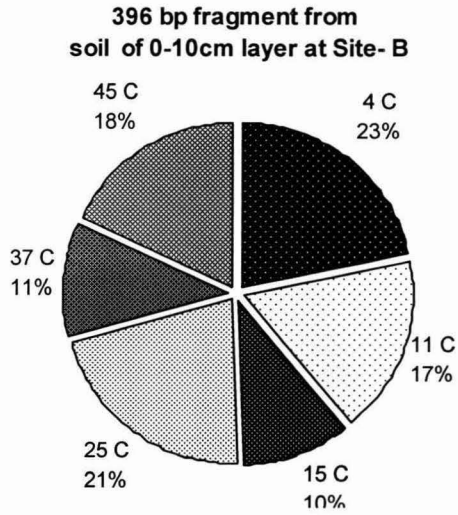
a)



b)



c)

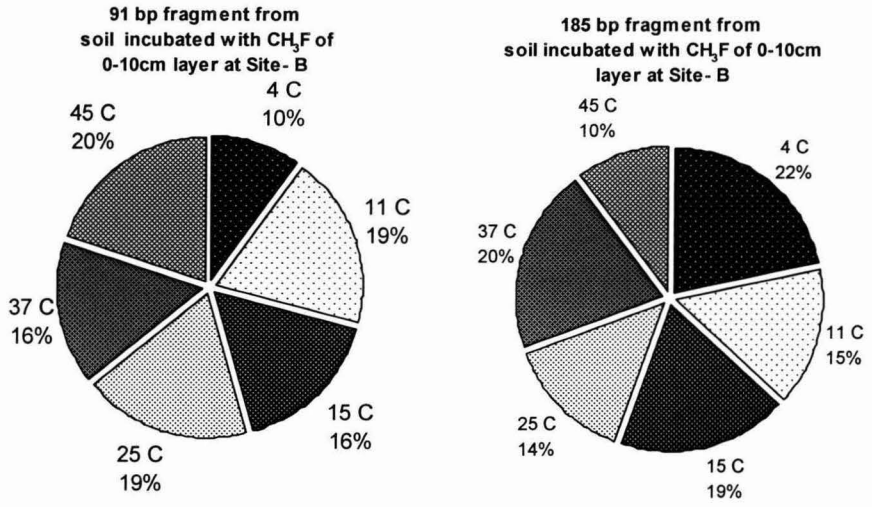


d)

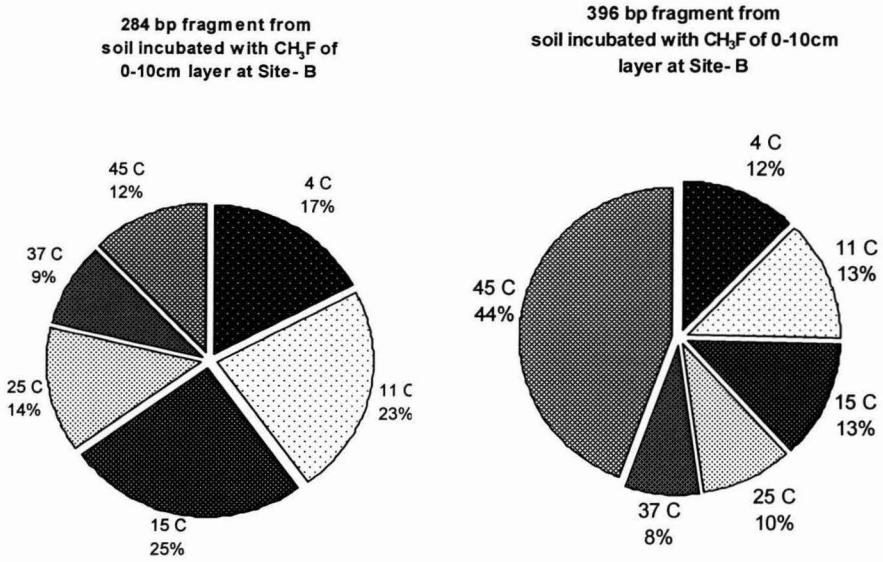
Fig. 38 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 0-10cm layer incubated at different temperatures at Site B

In the 0-10cm layer of soil, the relative fluorescence of 91 bp was (Fig.38a) highest at 11°C and lowest at 15°C. The relative fluorescence of 185 bp fragments (Fig.38b) was highest at 25°C and lowest at 15°C, The relative fluorescence of 284 bp fragments

(Fig.38c), was highest at 25°C and lowest at 15°C, The relative fluorescence of 396 bp fragments (Fig.38d) was highest at 4°C and lowest at 15°C. In the 0-10cm layer of soil incubated with methyl fluoride, the relative fluorescence of 91 bp fragments (Fig.39a) was highest at 45°C and lowest at 4°C. The relative fluorescence of 185 bp fragments (Fig.39b) was highest at 4°C and lowest at 45°C. The relative fluorescence of 284 bp fragments (Fig.39c) was highest at 15°C and lowest at 37°C. The relative fluorescence of 396 bp fragments (Fig.39d) was highest at 45°C and lowest at 37°C.



a) b)



c) d)

Fig.39 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 0-10cm layer incubated with methyl fluoride at different temperatures at Site B

In the 10-15cm layer of soil, the relative fluorescence of 91 bp fragments (Fig.40a) was highest 45°C and lowest at 15°C. The relative fluorescence of 185 bp fragments (Fig.40b) was highest at 45°C and lowest at 15 and 25°C. The relative fluorescence of 284 bp fragments (Fig.40c) was highest at 4°C. Fluorescence was not detected at 45°C. The relative fluorescence of 396 bp fragments (Fig.40d) was highest at 45°C and lowest at 15°C.

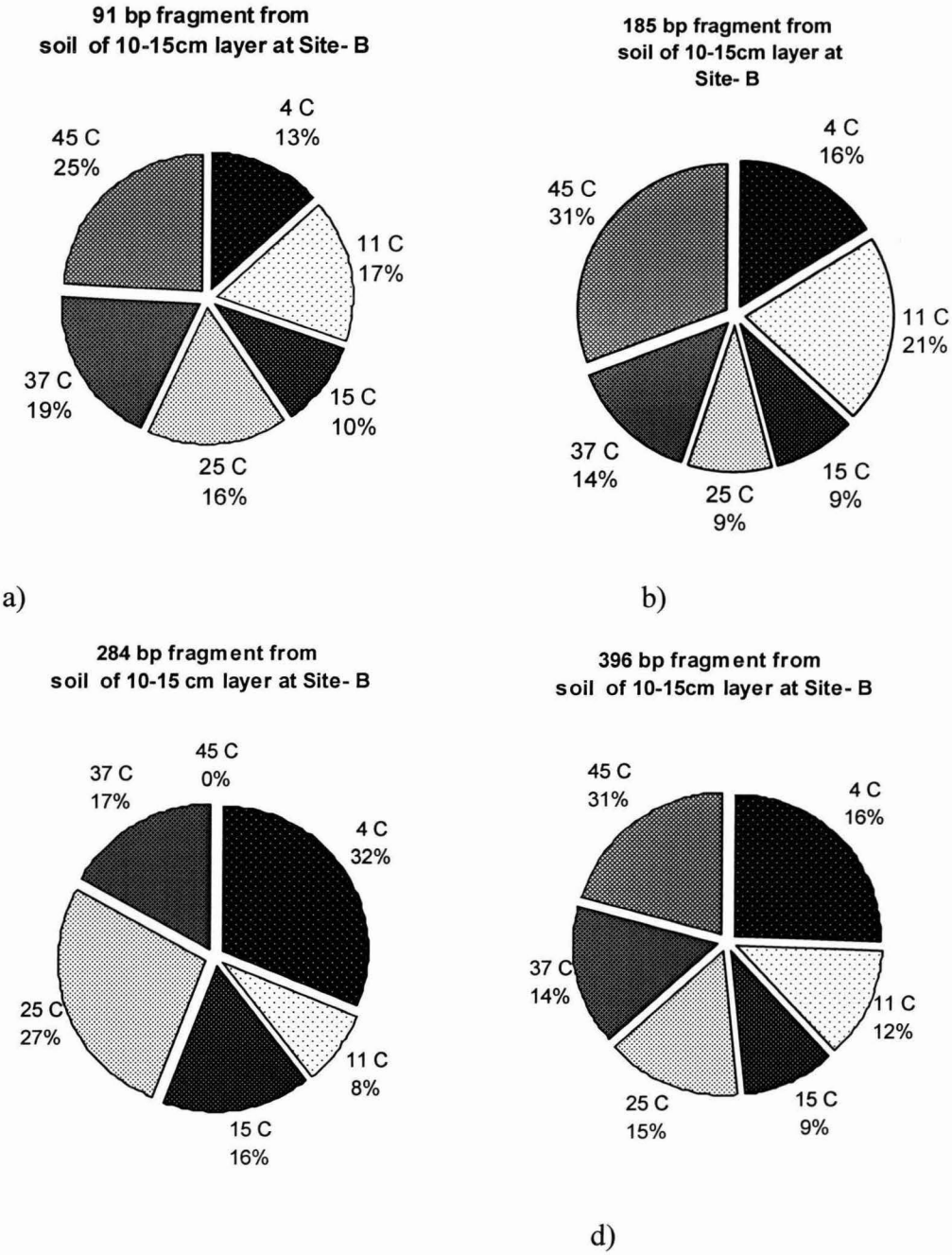


Fig. 40 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 10-15cm layer incubated at different temperatures at Site B.

In the 10-15cm layer of soil incubated with methyl fluoride, the relative fluorescence of 91 bp (Fig.41a) was highest at 45°C and lowest at 4 and 11°C. The relative fluorescence of 185 bp fragments (Fig.41b) was highest at 37°C. Fluorescence was not detected at 45°C. The relative fluorescence of 284 bp fragments (Fig.41c) was highest at 4°C Fluorescence was not detected at 45°C. The relative fluorescence of 396 bp fragments (Fig.41d) was highest at 4°C-26%. Fluorescence was not detected at 45°C.

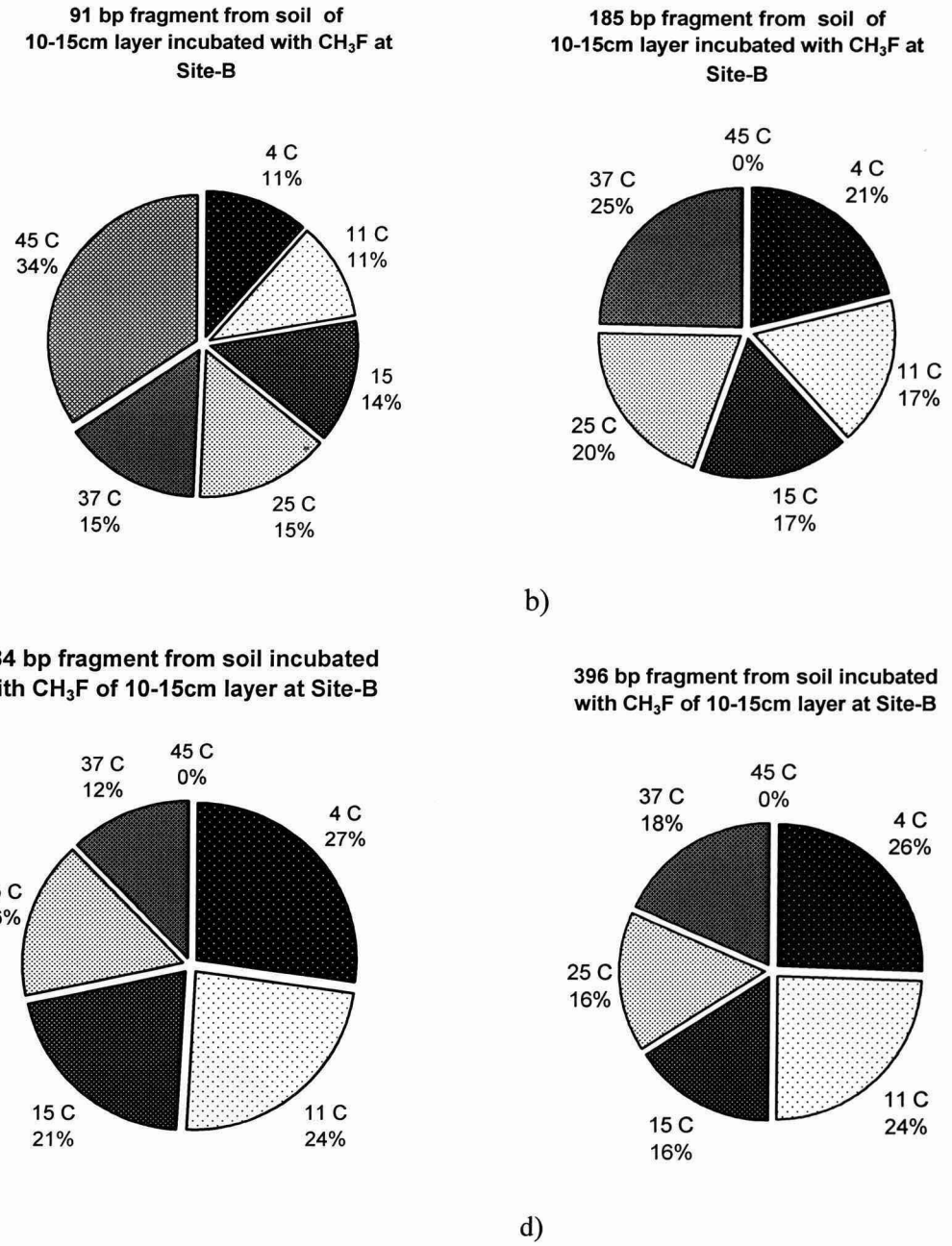


Fig.41 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil with methyl fluoride of 10-15cm layer incubated at different temperatures at Site B.

In the 15-20cm layer of samples, the relative fluorescence of 91 bp fragments (Fig.42a) was highest at both 15°C and 25°C. Fluorescence was not detected at 4 and 45°C. The relative fluorescence of 185 bp fragments (Fig.42b) was highest at 4°C. Fluorescence was not detected at 45°C. The relative fluorescence of 284 bp fragments (Fig.42c) was highest at 25°C. Fluorescence was not detected at 45°C. The relative fluorescence of 396 bp fragments (Fig.42d) was highest at 37°C. Fluorescence was not detected at 4, 25 and 45°C.

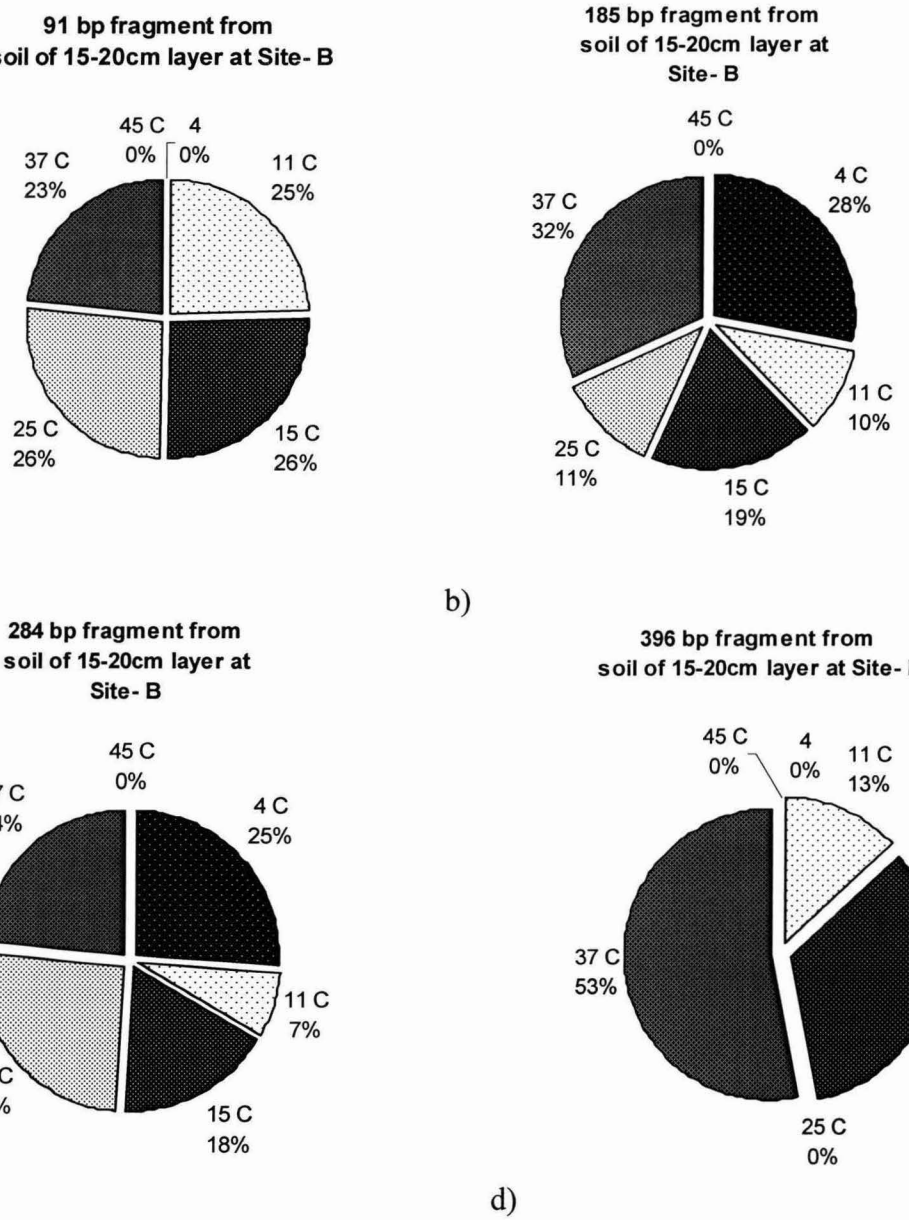
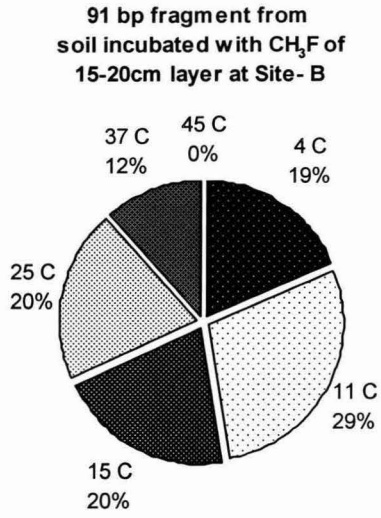
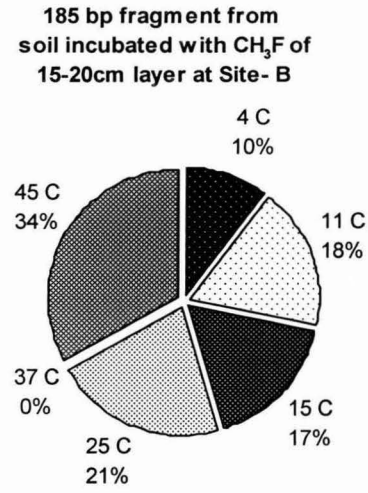


Fig.42 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 15-20cm layer incubated at different temperatures of Site B

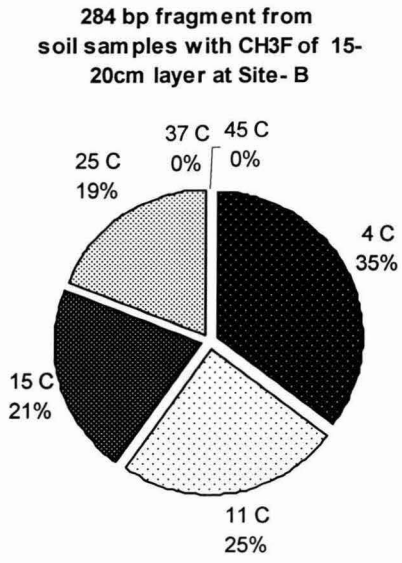
In the 15-20cm layer of soil samples incubated with methyl fluoride, the relative fluorescence of 91 bp fragments (Fig.43a) was highest at 11°C. Fluorescence was not detected at 45°C. The relative fluorescence of 185 bp fragments (Fig.43b) was highest at 45°C. Fluorescence was not detected at 37°C. The relative fluorescence of 284 bp fragments (Fig.43c) was highest at 4°C. Fluorescence was not detected at 37°C and 45°C. The relative fluorescence of 396 bp fragments (Fig.43d) was only found at 4°C.



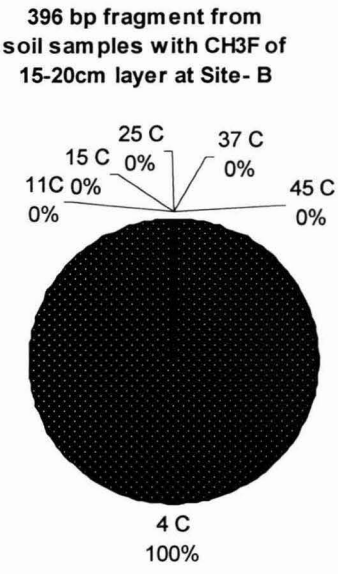
a)



b)



c)



d)

Fig.43 Variation in the relative fluorescence (%) of base pairs a) 91, b) 184, c) 284, d) 396 from soil of 15-20 cm layer incubated with methyl fluoride at different temperatures of Site B

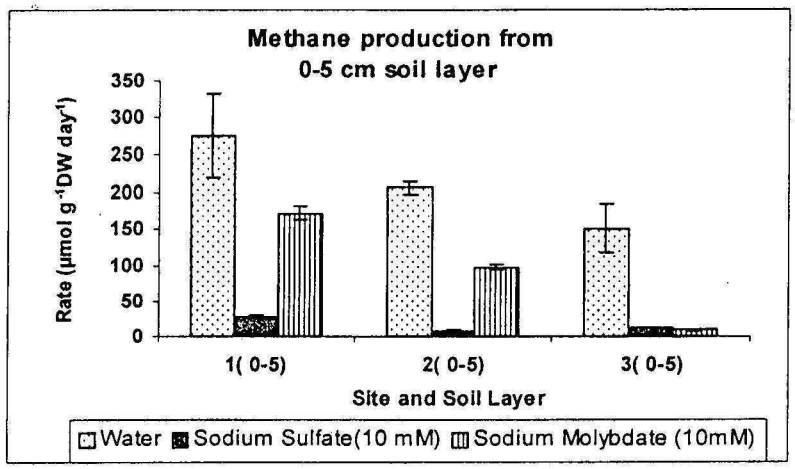
The response to the addition of alternate electron acceptors and substrates used by methanogens on methane production in Khajjiar Soil

Methane production was observed from the soils, collected from the three different depths in the three sites, incubated with competitors for substrates used by methanogens and substrates used by different groups of methanogens at 25°C. Soils incubated without any external additive acted as a control observation.

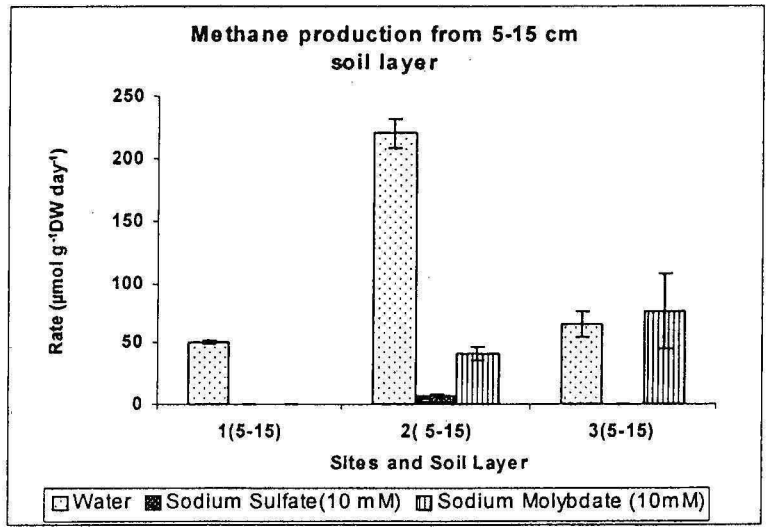
The soil from the 0-5cm layers at the three sites showed a similar trend in methane production on incubation with sodium sulfate (10mM), sodium molybdate (10mM) and sodium nitrate and for control (Fig.44a). The methane production was highest from all the three sites when incubated without any competitors for the substrate of methanogens. The rate of methane production in control was highest at Site-1 (275.08±56.66 (μmol g^{-1} DW day^{-1})) followed by Site-2 (205.77±9.02 (μmol g^{-1} DW day^{-1})) and at Site-3 (151.13±31.55 (μmol g^{-1} DW day^{-1}). The rate of methane production from control varied significantly from the rate of methane production when incubated with sodium sulfate and sodium molybdate from all the sites. The incubation with nitrate did not yield any substantial methane even after an incubation of 40 days at all the three sites. Sodium sulfate inhibited the production of methane by 90, 97 and 99% at Site-1, 2 and 3 respectively. The addition of molybdate increased methane production by 84, 92 and 85 % with respect to sulfate but also inhibited it by 35.88, 52.05 and 93.65 % with respect to control at Site 1, 2 and 3 respectively.

In the 5-15cm soil layer (Fig.44b), the potential rate of methane production was highest from the control and between the sites it was 219.44±11.72 at Site 2 followed by 69.92±7.59 at Site 3 and then 50.18 ±1.12 at Site 1. The % inhibition of the rate of methane production by sulfate was 99.61, 96.78 and 99.31 % at Site 1, 2 and 3 respectively. The addition of molybdate increased methane production by 16.50, 82.66 and 99.41 % with respect to sulfate but inhibited it by 99.53, 81.43 and 3.89 % with respect to control at Site 1, 2 and 3 respectively.

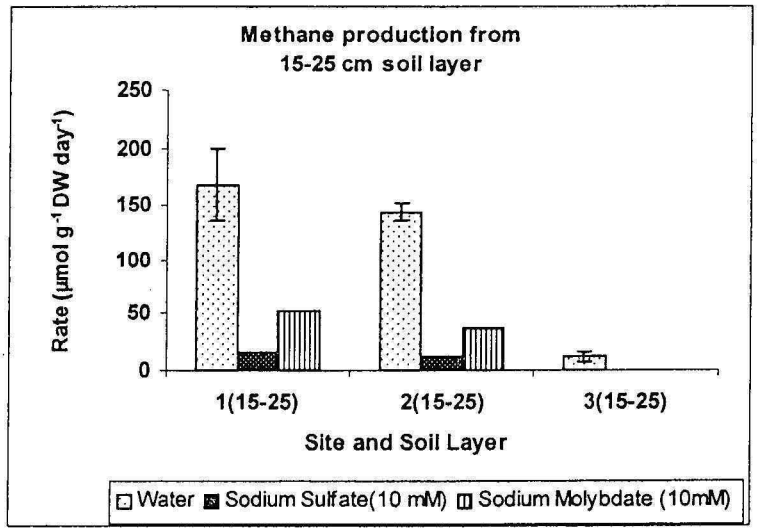
In the 15-25cm soil layers (Fig.44c), the potential rate of methane production was highest from the control and between the sites it was 167.97±4.21 at Site-1 followed by 143.94 ±14.36 at Site 2 and 11.45±4.39 at Site-3. The % inhibition by sulfate was 90.62, 90.98 and 100.31 % at Site 1, 2 and 3 respectively. The addition of molybdate increased methane production by 69, 65.65 and 100 % with respect to sulfate but inhibited it by 69.01, 73.96 and 98.52 % with respect to control at Site 1, 2 and 3 respectively.



a)



b)



c)

Fig.44 The variation in the rate of methane production (µmol g^{-1} DW day^{-1}) on incubation with or without external competitors for substrates used by methanogens from the a) 0-5 cm b) 5-15cm and c) 15-25cm of soil layers at three sites at Lake Khajjiar.

Stimulative responses due to the addition of substrate varied in the soil from three different depths and within the three sites (Fig.45). In the 0-5cm soil layers at Site 1 the stimulation in the rate of methane production by H_2/CO_2 was 133.13% whereas for acetate it was 75.68 %. At Site-2, the rate of methane production was inhibited by 5% and 30.21% on addition of H_2/CO_2 and acetate respectively. In the 5-15cm soil layer at Site-1, the stimulative response of H_2/CO_2 was 117.72 % but the addition of acetate reduced the rate of production by 53.15 %. At Site-2 the addition of H_2/CO_2 and acetate reduced the rate of methane production by 37.19 and 44.11% in the 5-15cm soil layer. At 5-15cm layer of Site-3, the addition of H_2/CO_2 and acetate stimulated the production by 37.31 and 100.29%. In the 15-25cm soil layers at Site-1 and 2 the addition of acetate and H_2/CO_2 had inhibitory effect on methane production whereas at Site 3 the addition of H_2/CO_2 and acetate stimulated methane production by 67.39 and 97.38% respectively.

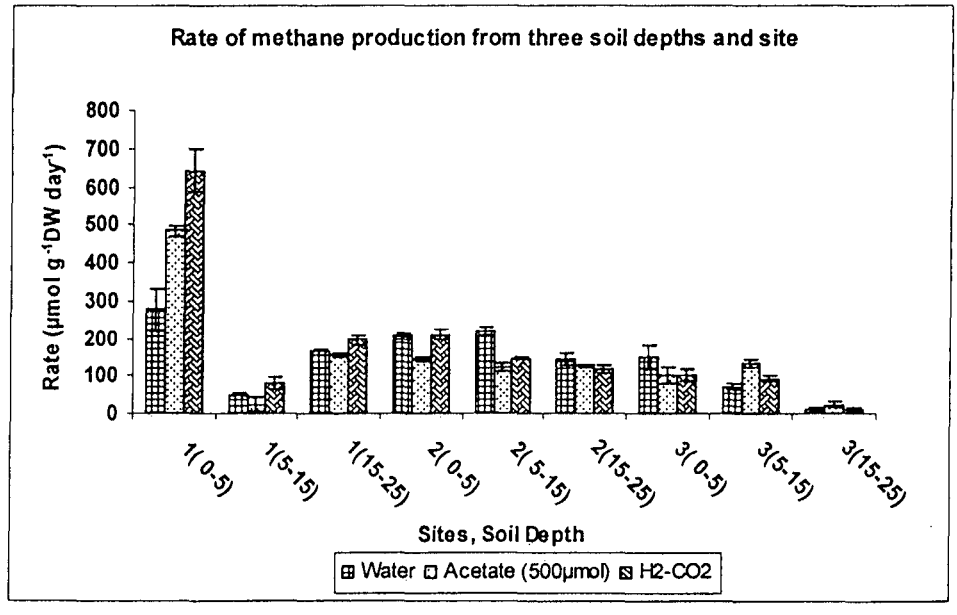


Fig. 45 The variation in the rate of methane production (µmol g^{-1} DW day^{-1}) on incubation with or without external substrates used by methanogens from the three soil depths at three sites at Lake Khajjiar.

Methane production from control was found to be significantly correlated to organic matter (Spearmann's nonparametric r^2=0.917, significant at p=0.001 level) (Fig.46).

There was a positive but a low correlation between methane production from control and microbial carbon and microbial biomass (Spearmann's nonparametric r^2=0.429) (Fig.47)

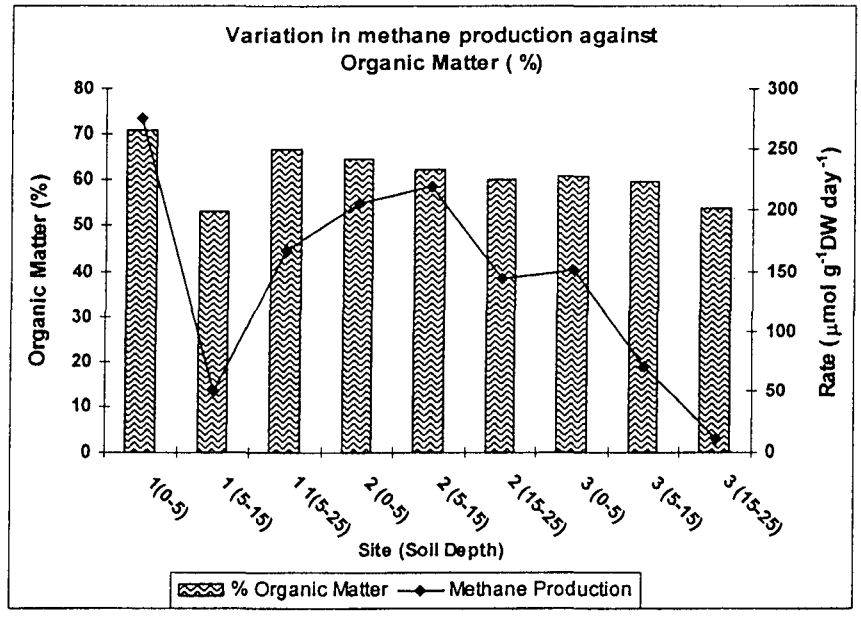


Fig.46 The variation in the rate of methane production (µmol g^{-1} DW day^{-1}) in relation to organic matter (%) from three depths of the three sites.

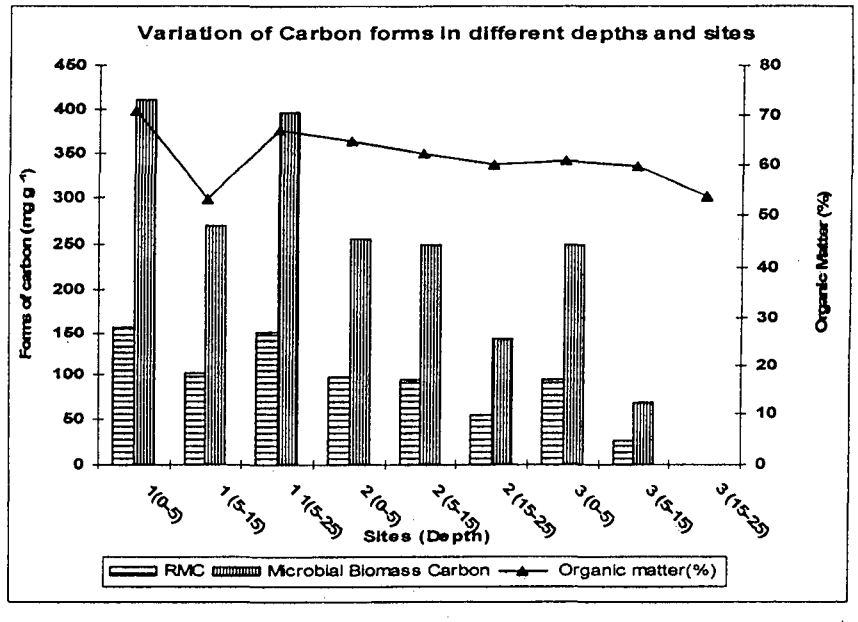


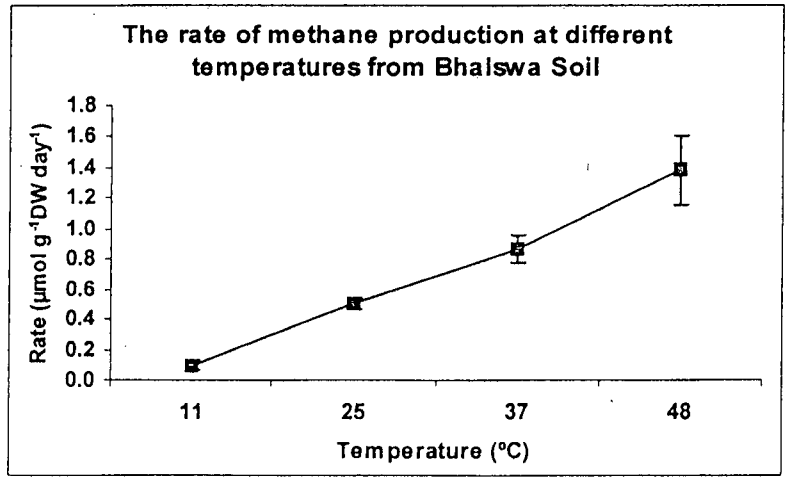
Fig.47 The variation in the rate of methane production in relation to microbial carbon and biomass from three depths of the three sites.

Methane Production from Natural Wetland containing Mineral Soils

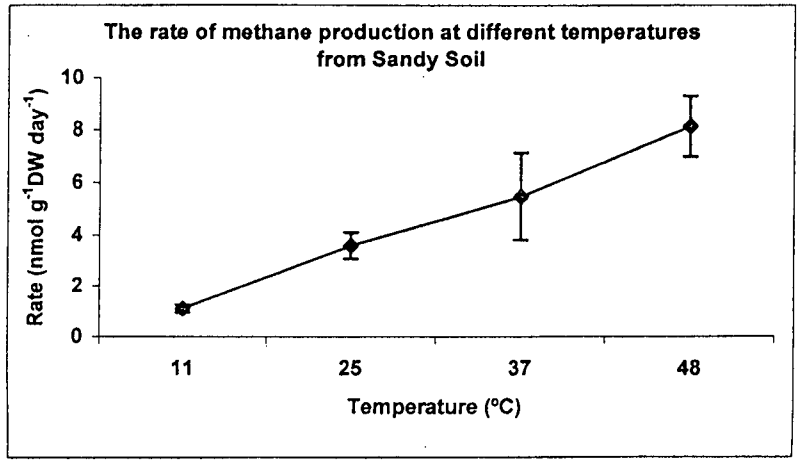
The potential methane production of soils was observed by incubating the soils at four different temperatures.

The rate of methane production increased linearly with the rise in temperature in Bhalswa soil (Fig.48.a). Potential methane production was observed at 48°C.
The rate of methane production increased linearly with the rise in temperature in the sandy soil (Fig.48.b). Potential methane production was also observed at 48°C.
In the garden soil (Fig.48.c) the rate of methane production hardly increased till 37°C and then it shot to 3.080±1.07 μmol g^{-1} DW day^{-1}at 48°C. Potential methane production was also observed at 48°C.

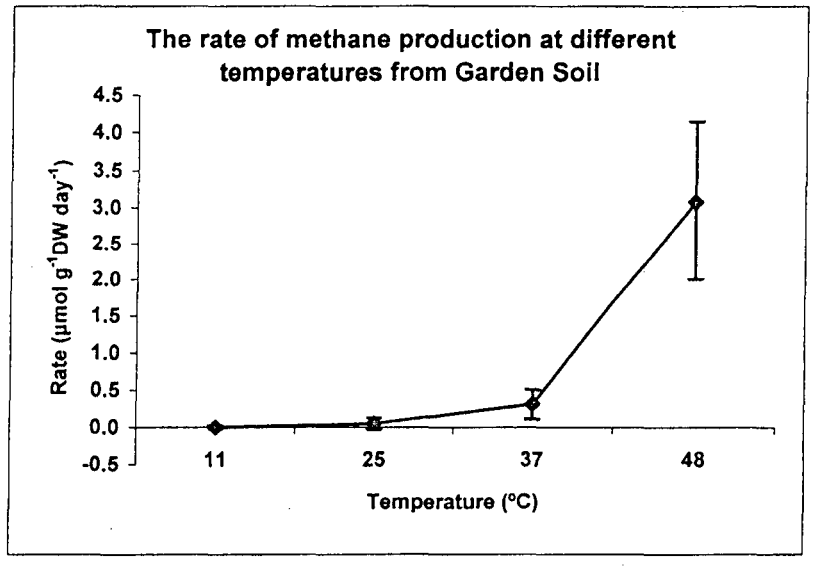
A comparison between the potential rate of methane production at 48 °C from different soils shows that the garden soil had the maximum rate of methane production followed by Bhalswa Soil and then Sandy soil.



a)



b)



c)

Fig.48. Rate of methane production at four different temperatures from a) Bhalswa soil, b) Sandy soil and c) Garden soil

The response to the addition of competitors and substrates used by methanogens on methane production in Bhalswa Soil

The rate of methane production varied on incubation with different inhibitors at 25 °C from soil of Lake Bhalswa (Fig.49). The addition of sulfate reduced the rate of methane production by 54% and the addition of molybdate reduced the rate by 7%. With respect to sulfate the addition of molybdate recovered the rate of methane production by 50%.

The rate of methane production of soil from Lake Bhalswa also varied on incubation with different substrates at 25 °C (Fig.50). The addition of 0.5 M sodium acetate significantly stimulated rate of methane production to 52.74±1.76 µmol g^{-1} DW day^{-1}. The rate increased by 51 times compared to the control. The addition of H_2/CO_2 inhibited the rate of methane production. The rate reduced by 0.58 times compared to the control.

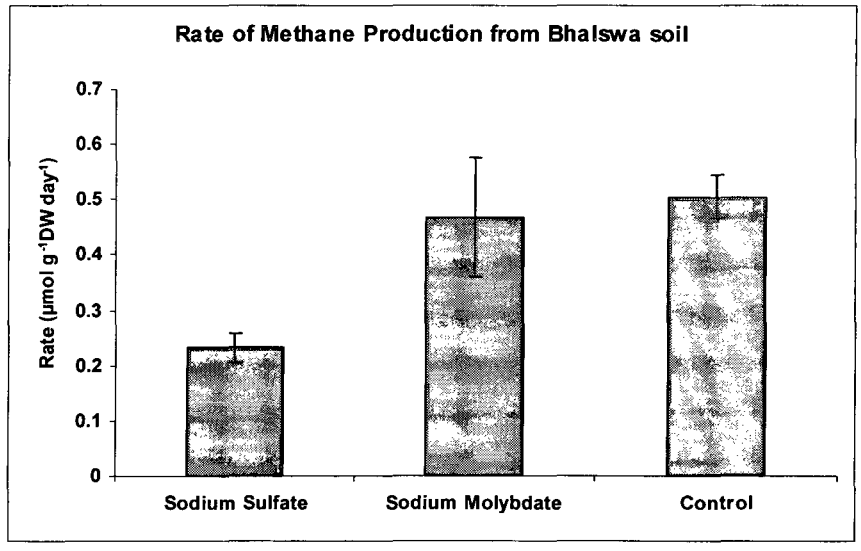


Fig.49. The variation in the rate of methane production (µmol g^{-1} DW day^{-1}) on incubation with or without external competitors for substrates used by methanogens from soils at Lake Bhalswa

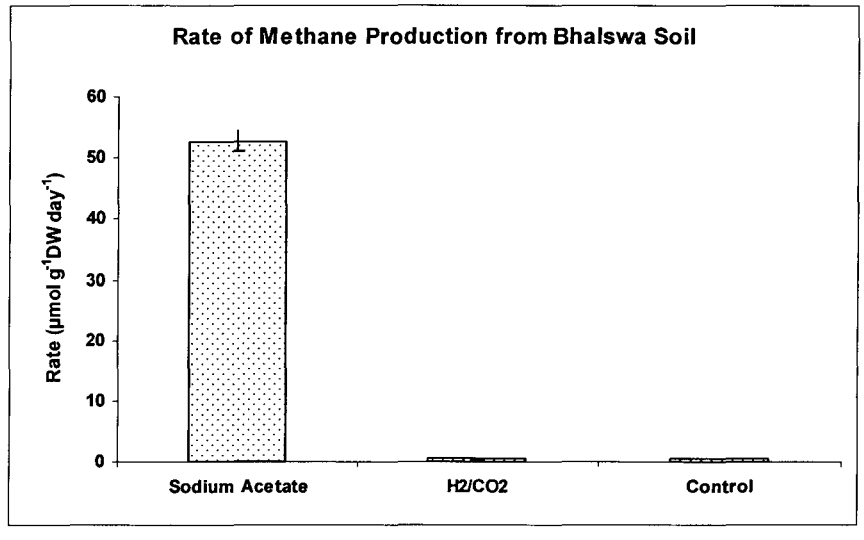
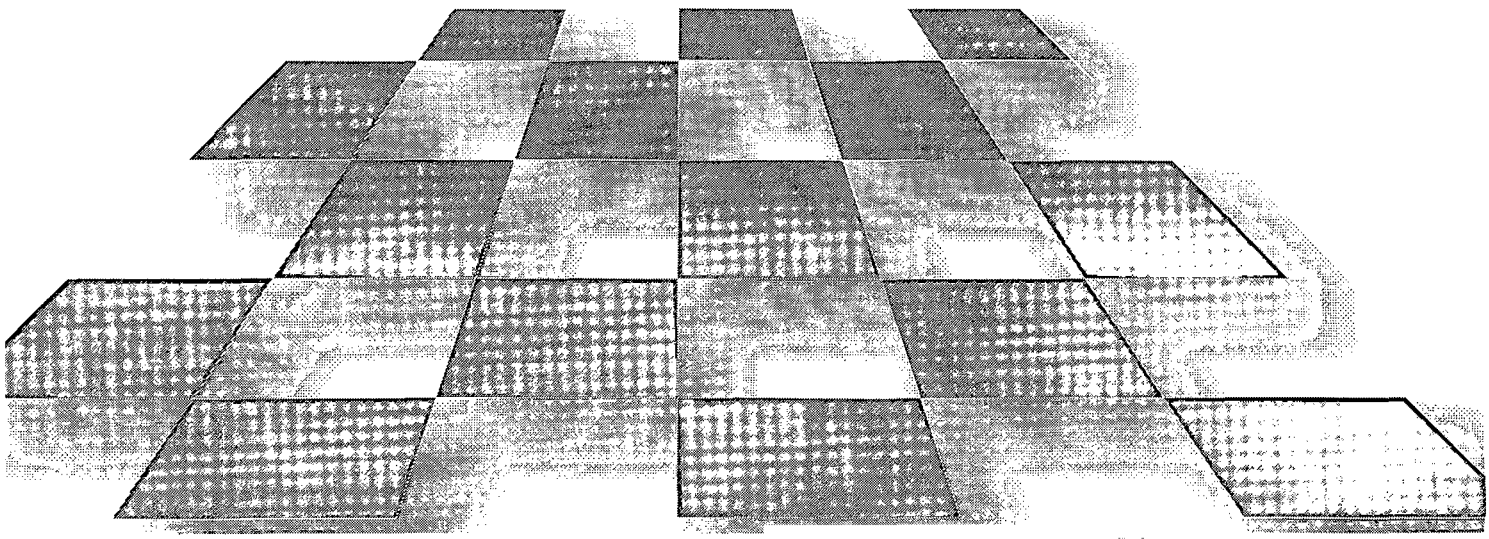


Fig.50. The variation in the rate of methane production (µmol g^{-1} DW day^{-1}) on incubation with or without external substrates used by methanogens from soils at Lake Bhalswa.

DISCUSSIONS



Chapter V

Discussion

Methane Emission from Natural Wetlands

The mean rate of methane emission from bare soil at Site-1, 2 and 3 at Lake Khajjiar (collected in 2005) were 0.37±0.21, 0.30±0.11 and 0.37±0.33 mg $m^{-2} hr^{-1}$, respectively (Fig. 6) The spatial variability of CH_4 flux is often high, even within sites of the same wetland (Schimel, 1995) and also ecologically similar wetlands (Moore and Knowles, 1990; Morrissey and Livingston 1993). But there was no significant difference in the rate of methane emission between the sites at Lake Khajjiar. Methane emissions ranged from 10 mg CH_4 $m^{-2} d^{-1}$ at a forested bog hummock to 180 mg $m^{-2} d^{-1}$ at an open poor fen in Minnesota peatlands (Dise, 1992).

Average mid day methane emission from Mc Lean bog and Labrador Hollow bog in New York was 11152± 2392 ng $m^{-2} s^{-1}$ and 368±88 ng $m^{-2} s^{-1}$ from May 1993 to 1995. Mean methane emission rates from lawn and mud bottoms from Central Siberia were 128±23 and 106±31mg $m^{-2} d^{-1}$ (Bosse and Frenzel, 2001).

The rate of methane emission ranged from <0.16 to 52.8 mg $m^{-2} hr^{-1}$ in winter to 44 mg $m^{-2} hr^{-1}$ in summer at the Ryans-1 Billabong, Australia (Boon and Mitchell, 1995) due to the strong dependence of methanogenesis to temperature fluctuation.

Role of plants in methane emission

Vegetation plays a major role in methane emission from wetlands (Schimel, 1995; Greenup et al., 2000). Plants may ventilate upto 90% of methane produced in the anoxic soil (Dacey and Klug, 1979; Cicerone and Shetter, 1981; Whiting and Chanton, 1992; Morrissey et al., 1993) but this ventilation capacity is species specific (Shannon and White, 1994). At Lake Khajjiar, the rate of methane emission was found to vary significantly amongst the dominant plant species (Fig.6). The rate of methane emission from bare soil was higher than the methane emission from the dominant in-situ plant species *Acorus, Phragmites* and *Cyperus* present at Site-1 and 2 but not more than that from *Phragmites* at Site 3 at Lake Khajjiar.

Emergent plants that possess convective throughflow mechanisms are many times more efficient in methane emission than species that possess diffusion mechanism only (Brix et al., 1992). *Cyperus* sp. employ diffusion mechanism for the transport of gases and hence their capacity to emit methane is low compared to the *Acorus* and *Phragmites* sp. that use pressure-induced convective throughflow mechanism for the gas transport. Though they employed the same mechanism for gas transport yet *Acorus* and *Phragmites* sp. differed in their rate of methane emission despite growing in the same site. The difference in the rate of emission maybe due to the high aboveground biomass of the *Acorus* sp at Site 1. Similar reasons were cited for the difference in the flux capacity from three different plant species that used the same gas transport mechanism but differed in their biomass in the Luanhaizi wetlands (Hirota et al., 2004). The higher methane efflux rate seen in *Phragmites* sp at Site 3 suggests that the positive effects of photosynthate provision and methane transport outweighed the effects of oxygen inhibition of methanogens and oxidation of methane by methanotrophs.

The rate of methane emission from *Phragmites* (0.18±0.025 to 1.33±0.93 mg m^{-2} day^{-1} at Site 1 and 3) were far less than the rate observed elsewhere. The rates reported in terms of mol CH_4 m^{-2} d^{-1} by other workers has been converted to mg CH_4 m^{-2} d^{-1} after multiplying with molecular weight of methane i.e. 16.

The rate of emission from *Phragmites* sp. in July was 15.5 to 80.0 mg CH_4 m^{-2} hr^{-1} in Lake Vesijaervi, southern Finland (Kaeki et al., 2001).

At the two sites of tidal freshwater marsh, the rate of methane emission from the *Phragmites* species ranged from 54.08±11.84 to 310.4±19.52 mg m^{-2} day^{-1} in the light, from 53.12±12.8 to 212±24 mg m^{-2} day^{-1} at dark (plants subjected to 3-4 weeks of darkness) from Site 1. The average rate of methane emission from *Phragmites* sp. at Site- 2 was 19.2±1.6 to 24±1.6 mg m^{-2} day^{-1} to 134.4±0.96 at dark (plants subjected to 3-4 weeks of darkness) (Van der Nat and Middelburg, 1998).

The mean methane emission from two plots vegetated by *Phragmites* plants showed a mean methane emission of 17.93±8.77 mg m^{-2} hr^{-1} from the plot with higher water

depth compared to 12.67±6.18 mg m^{-2} hr^{-1} from the plot with lower water depth (Duan et al., 2005).

Mean daily methane fluxes ranged from 0.6-68.4 mg m^{-2} day^{-1} in plant communities dominated by *Chamaedaphne calyculata* to 11.5-209 mg m^{-2} day^{-1} dominated by *Carex oligosperma* and *Scheuchzeria palustrisin* two peatlands (bogs) in Michigan. The difference is because the former is an ericaceous shrub and the latter two plants are aerenchymatous nature (Shannon and White, 1994).

The rate of methane flux from densely populated site of *Eriophorum angustifolium* was 8080±1072 μg m^{-2} hr^{-1} and 1344±1888 μg m^{-2} hr^{-1} from *Sphagnum* dominated site of Northern Wetlands (Thomas et al., 1996).

The mean rate of methane emission reported from *Sphagnum* lawns was 34 μg m^{-2} hr^{-1} and *Eriophorum vaginatum* was 974 μg m^{-2} hr^{-1} from a bog in Estonia. *Eriophorum angustifolium* that possess diffusive transport mechanism was observed to emit methane at a rate of 5.5 mg m^{-2} hr^{-1} and formed the major pathway for methane fluxes from the above bog (Frenzel and Rudolph, 1998).

Methane flux reported from the tillers of *Eriophorum angustifolium* and *Carex aquatilis* are 9.5±1.5, 5.0±1.8 μg hr^{-1} respectively, from a wet meadow of Alaska. The difference in the rate of emission is due to the difference in the size and structure of two species (Schimel, 1995).

The mean rate of methane emission was significantly higher from *Equisetum limosum* dominated grassland (165.5 mg m^{-2} d^{-1}) compared to the *Carex wiluica* dominated grassland (73.2 ± 4.9 mg m^{-2} day^{-1}) from North-Eastern Siberia (Tsuyuzaki et al., 2001).

Carex oligosperma and *Chamaedaphne cayculata* were found to emit 0.22 mg CH_4 g^{-1} DW day^{-1} and 0.09 mg CH_4 g^{-1} DW day^{-1} from the Big Cassandra Bog.

Methane emission from *Carex acuta* and *Carex disticha* dominated fresh water wetlands in Belgium were 0 to 102 mg m^{-2} day^{-1} from March until December. Soil

moisture was the major controlling factor than the soil temperature in the above-vegetated sediments (Boeckx and Cleemput, 1996).

Chanton et al., (1993) reported mean rate of methane efflux from *Typha* plants were 1.65μg m^{-2} s^{-1} from wetlands in Florida whereas Yavitt and Knapp, (1997) reported 0.75 to 9.2 μg m^{-2} s^{-1} from different wetlands in Ithaca. Sebacher et al., (1985) reported a flux of 2.96 μg m^{-2} s^{-1} from *Typha* plants after single sampling.

Scirpus plants played a dominant role in methane flux from mineral soils. The rate of methane emission from *Scirpus* plants grown in Bhalswa, Sandy and Garden soil exceeded the rate of methane emission from the unvegetated soil (Fig. 14,15 and 16). The dominance (%) of methane emission (with respect to bare soil) by *Scirpus* plants varied across the soil types and also months. The dominance of *Scirpus* plants varied from 66 to 93 % from May to October in Bhalswa soil, 52 to 76% from May to October in Sandy soil and 28 to 76% from June to July in the Garden soils. With the exception of occasional upsurge (72%) of soil efflux from bare Garden soil in October, methane efflux did not exceed the flux from the vegetated soil.

Significant diel variation in the rate of methane emission was observed from the *Scirpus* plants grown in three types of soils (Bhalswa, Sandy and Garden). *Scirpus* plants are one-way (diffusive) transport species and known to be less sensitive to change in light condition. Diel differences in the rate of methane emission were low in these plant species (Van der Nat et al., 1998). Clear patterns of diel variation in methane emission were observed only (at the end of growing season) from plants like *Phragmites australis* and *Typha latifolia*. The diel pattern was found to depend upon the plant growth stages of the above-mentioned plants (Kim et al., 1999; Kaeki et al., 2001).

A significant peak that coincided with the sunrise was observed in the rate of methane emission from *Scirpus* plants irrespective of the soil type in which they were grown. A small peak following incident light in *Scirpus* plants was also observed earlier (Van der Nat et al., 1998). The plausible explanation was the heating of the plant tissue by light leading to higher methane emission. The opening of stomatal aperture with the incident light is the main reason for the peak of methane emission (Van der Nat et al.,

1998). The peak methane emission was not reported to be significant but Hirota et al., (2004) observed a clear diurnal change in methane flux from *Scirpus* plants that correlated with light intensity. The micropores reported on the tiller surface, act as vents for methane to escape (Kulshrestha et al., 2000) and could be the reason for the more or less same rate of methane emission between the day and night time.

The rate of methane emission from bare soils (Bhalswa, Sandy and Garden) did not vary significantly within the four months (Fig.18). This could be due to the absence of vegetation in these soil. Plants contribute to higher incorporation of litter carbon, mainly into microbial biomass. This is connected with the higher amount of microbial biomass in the presence of living plants and higher incorporation of litter C into growing microbial cells. Thus plants favour the rhizospheric community (Bonkwosky et al., 2004) of which methanogens are a dominant group in the anoxic soil.

The mean daily rate of methane emission ranged from 5.38±2.25 mg m^{-2} day^{-1} in May to 3.90 mg $m^{-2}day^{-1}$ in October from *Scirpus* plants grown in Bhalswa soil (Fig.19). The mean rate of emission ranged from 0.478±0.082 mg m^{-2} day^{-1} in May, 1.55±0.48 in June to 0.216±0.05 mg m^{-2} day^{-1} in October from plants grown in Sandy soil. The mean rate of emission ranged from 35.04±7.71 mg m^{-2} day^{-1} in June to 2.297±0.37 mg m^{-2} day^{-1} in October from *Scirpus* plants grown in Garden soil.

The average rate of methane emission from *Scirpus* plants at Site 1 of tidal freshwater marsh ranged from 20.8±12.64 to 97.44±10.88 mg m^{-2} day^{-1} in light and ranged from 6.72±3.84 to 90.88±14.72 mg m^{-2} day^{-1} at dark. The rate of emission from *Scirpus* plants at Site 2 ranged from 42.72±9.12 to131.36±6.4 mg m^{-2} day^{-1} in light and 36.32±1.6 to 111.36±7.52 mg m^{-2} day^{-1} at dark (Van der Nat and Middelburg, 1998a). The seasonal methane flux reported from the *Scirpus distigmaticus* L. plants in Luanhaizi wetlands, China range from 25.4-156 mg m^{-2} day^{-1} (Hirota et al., 2004).

Methane Emission and Soil Types

The physicochemical characteristic of soil does play a major role in methane emission. The rate of methane emission from *Scirpus* sp. varied when grown in soils differing in texture and organic matter. The rate of methane emission from *Scirpus* plants grown

in Garden soil was significantly higher than Bhalswa soil and Sandy soil. Sandy soils with low water holding capacity produced and emitted less methane as compared to the organic soils (1 versus 55mmol m^{-2} d^{-1}) (Gruenfeld and Brix, 1998). A strong correlation was observed between the organic matter content and the total methane emission from the *Scirpus* plants in Bhalswa soil (r^2=0.944) (Fig. 20). The rate of breakdown of soil organic matter was also influenced by the soil pH (De Laune et al., 1981) and by the clay content in incubation experiments (Saunder and Grant, 1962; Delphin, 1988; Simard and N'dayegamiye, 1993). Earlier studies showed that the methane entrapped in the soil was positively correlated with soil clay content. Therefore soils with heavy clay content may have large potential for methane entrapment (Wang et al., 1993). The reason for the stable rate of methane emission over the four months from the *Scirpus* plants grown in Bhalswa soils may be due to the high clay content (31.31±0.66%) (Table 6.) in these soils that ensured a regular supply of substrates by mineralization and a gradual release of gas that may be entrapped by the clay.

Methane Emission and Plant growth

Weak correlation between the biomass and total methane emission was found in the *Scirpus* grown in Bhalswa Soil (Fig.22). This is because the mean daily rate of methane emission was very stable over the four months. So the plant productivity is not the only factor that contributes to the methane production. A significant correlation was observed between the total methane emission and biomass of *Scirpus* plants grown in Sandy soil (r^2=0.79) and Garden soil (r^2=0.88). Rate of methane emission from senescing *Scirpus* plants grown in Garden soil and Sandy soil decreased in October. A linear correlation was observed between methane flux and plant biomass in the Everglades *Cladium* sp. marsh (Whiting et al., 1991). Methane fluxes increased with the above ground biomass of plants in the Luanhaizi wetlands (Hirota et al., 2004). The maximum emission was observed in the tillering stage and the minimum in the senescent stage of *Phragmites* plants (Chanton and Dacey, 1991; Kaeki et al., 2001).

Methane Emission and Water Depth

Wetland plants are subjected to dynamic water level changes that influence their methane emission capacity. Methane emission from wetland plants depends on the duration of flooding and change in water level (decrease and increase in the height and depth of water above or below the soil surface) (Fig. 23). The rate of methane emission from the *Scirpus* plants increased gradually with duration of flooding. Lag phase was observed in methane emission from shallow (15 cm) submerged plants but not in the deeper (30 cm) submerged plants (Experiment in tanks). The rate of methane emission from deeply submerged *Scirpus* plants (30 cm) was significantly higher than the shallow submerged *Scirpus* sp. This observation differed from earlier works where the rate of emission reduced with higher submergence (Orawan and Suphasuk, 2002; Juutinen et al., 2003) of plants. Juutinen et al., (2001) observed that the rate of methane emission was low during the high water level (> 40cm above ground), reached the maximum value when the water level was near the soil surface, and decreased again when the water level dropped considerably (< 15 cm in mineral soil). The plausible explanation for the reduced methane emission was the submergence of the vents of the plants through which gases diffused outside (Juutinen et al., 2003). The concentration of methane emission from *Scirpus* plants, which grew on soils with the water table below the soil surface, was equal or less than the atmospheric concentration of methane. The lowering of water table could be related to situations when wetlands dry up temporarily, exposing the soil to oxygen. As methanogens are obligate anaerobes, their viability reduces when the water table drops. Other than that the drying of soil moisture also induces stress on primary production and reduces the abundance of labile organic substrates used by methanogens (Dowrick et al., 2006). In boreal peatlands, spatial and temporal differences in methane emissions have been related to water table level; the higher water table level is generally associated with higher methane emissions (Bubier, 1995; Saarnio et al., 1997). Conditions in littoral wetlands however, differ from those in peatlands, because the changes in water level and water depth are very high. In ephemerally flooded wetlands, the methane emissions are largely associated to water level (Boon et al., 1997; Juutinen et al., 2001). The pattern of water level fluctuation is reflected in methane fluxes, which are low in intermittently flooded soils and vice versa (Pulliam and Meyers, 1992; Otter and Scholes, 2000; Juutinen et al., 2001).

Methane emission is a resultant of methane production and methane oxidation. Methane oxidation by methanotrophs lodged on the plant surfaces, rhizospheric zone as reported earlier (King et al., 1990; Bosse et al., 1993; Gerard and Chanton, 1993; King, 1994). Methane consumption was observed in all the plant parts as well as rhizospheric soil (Table 9.). The maximum methane consumption was observed in roots followed by rhizomes, shoots (till 36 cm plant length from the base) and then rhizosperic soil. The belowground tissues of aquatic plants function as a dynamic oxygenated biofilter that facilitates methane consumption. Methane oxidation from the rhizosphere of *Scirpus* plants reduced the potential methane flux by 34.7±20.3%. The rhizospheric region of *Scirpus* plants were found to be more active than *Phragmites* plant and the rate of oxidation decreased with the plant growth cycle (Van der Nat and Middelburg, 1998b). The rhizoplane, and likely the interior, of the roots and rhizomes of aquatic vegetation promote the growth of methanotrophs, thereby substantially expanding the volume of the methanotrophic zone below the sediment surface (King, 1994). Methane consumption was found to be higher in the rhizoplane than in the rhizosphere of the rice plants (Gilbert and Frenzel, 1995). The high activity of methanotrophs observed in the primary roots of *Scirpus* plants in this study is quite possible as the oxygen is released though them. Low methane consumption in the rhizospheric soil may be due to the relatively anoxic condition in that region compared to the root surface and the shoot. Methane consumption by the shoots of *Scirpus* sp. indicates that substantial quantity of methane that is released from the plants is consumed at the surface. This could also explain the higher rate of methane emission from *Scirpus* sp. that were inundated to a higher depth compared to those that were less inundated.

Methane Production at different temperatures from Natural Wetlands

The rate of methane production decreased along the vertical gradient of soil core 0-10, 10-15 and 15-20cm at Lake Khajjiar (Fig. 24 and 27). Similar trend of methane production was also observed in soil cores from other wetlands (Kelly and Chynoweth, 1980; Zeikus and Winfrey, 1976; William and Crawford, 1984; Boeckx and Cleemput, 1997). The rate of methane production from peat soil at Lake Khajjiar was higher than mineral soil at Lake Bhalswa.

The optimum temperature for maximum methane production from the soil layers 0-10, 10-15 and 15-20 cm at Site A and B was 37 °C at Lake Khajjiar. This optimum temperature is far higher than the in-situ (annual average range ~2 and 25.5°C) temperature experienced at any time of the year. The optimum temperature for maximum rate of methane production was 48°C in the mineral soils (soil from Lake Bhalswa, Sandy soil and Garden soil). The optimum temperature of 48°C for maximum methane production in mineral soils of Lake Bhalswa is higher than the 37°C found in peat soil from Lake Khajjiar. It is often observed that the optimum temperature at which there is maximum rate of methane production is higher than the in-situ temperature experienced throughout the year in the peat soil and lake sediments (Metje and Frenzel, 2005; Zeikus and Winfrey, 1976; Boon and Mitchell, 1995). The in-situ temperature of an acidic peat from a mire in northern Scandinavia was lower than the optimum temperature of 25°C at which maximum methane was produced (Metje and Frenzel, 2005). At Lake Mendota, Wisconsin, the optimum temperature of 35°C to 42°C for maximum methane production from the incubated sediments was considerably higher than the maximum in-situ temperature of 23°C (Zeikus and Winfrey, 1976). In the sediments of Ryans-1 Billabong, Australia, the methane production was highest at 40°C and lowest at 5°C (Boon and Mitchell, 1995). Dunfield et al., (1993) found that the optimum temperature for maximum methane production was 25°C from peat soils. Wagner and Pfeiffer, (1997) found two temperature optimas for methane production in sediments of Elbe river. This observation was supported by the study of Svensson, (1984), who found that the temperature optima of the two groups of methanogens have different peaks of methane production, the group that uses acetate has a peak at 20°C and the other group that uses carbon dioxide has a peak at 28°C.

Carbon dioxide is also released during fermentation processes other than methanogenesis (Fig. 25 and 28). At Lake Khajjiar, the rate of production of carbon dioxide differed with depth at Site A. In the 0-10 cm, the rate of production was more or less equal to that of methane. For the other two depths (10-15 and 15-20cm), until 15°C, the production rate of carbon dioxide was significantly high compared to methane. At higher temperature the difference in the rate of production between carbon dioxide and methane decreased. At lower temperatures, the rate of production of carbon dioxide was more than methane at three depths of soil layers 0-10, 10-15

and 15-20cm at Site B. It became more or less equal at the optimal temperature but the differences increased with the rise in temperature as the rate of production of methane surpassed carbon dioxide production.

An increase in temperature until the optima (37°C) resulted in increased carbon mineralization (sum of produced methane and carbon-dioxide) in the peat soil of Khajjiar (Fig. 25 and 28). Rate of organic carbon mineralization was 0.60 to 15.34, 0.75 to 6.28, 0.83 to 7.51 μmol (CH_4+ CO_2)-C g^{-1} DW day^{-1} from 4°C to 37°C at 0-10cm, 10-15cm and 15-20cm layer at Site A. Rate of organic carbon mineralization was 2.08 to 16.95,0.95 to 11.09, 0.38 to 4.87 μmol (CH_4+ CO_2)-C g^{-1} DW day^{-1} from 4°C to 37°C at 0-10cm, 10-15cm and 15-20cm layer at Site B.

At site A, fermentative processes other than methanogenesis was active at lower temperatures until 15°C (RI=1) at the 10-15 and 15-20cm (Fig.25). With the rise in temperature methanogenesis became the dominant process. At Site-B (Fig.28), methanogenesis was the dominant process at all the temperatures across the depth of soil except at 4°and 11°C of the 15-20cm layer of soil (RI=0.99 and 0.8).

Rate of methane production from different layers of soil cores at Lake Khajjiar was studied in the second consecutive year (Fig.44). The rate of methane production from the soils collected in 2005 was two times higher than from the soil collected in 2004. This difference was observed in all the layers of soil cores between the different sites sampled in 2004 and the sites sampled in 2005. The soil was sampled in June 2004 which is the peak summer season and in August-September 2005, which is the rainy season. This difference in season is reflected significantly in the organic matter content of the soil and hence could be the major influencing factor for the difference in rate of methane production.

Dominant pathway of methane production

The inhibitory action of the acetoclastic methanogenesis by the addition of methyl fluoride gives an insight to the possible (hydrogenotrophic) pathway of methane production. Methane was produced from the peaty soils of Lake Khajjiar from hydrogenotrophic as well as acetoclastic methanogenesis. The production of methane

from acetoclastic methanogenesis was dominant at all temperatures in the three soil layers 0-10cm, 10-15cm and 15-25 cm at Site A (Fig.30a). The dominance of acetotclastic methanogenesis varied from 50 to 83%, 50 to 71% and 90 to 77% at different temperatures, in soil from the 0-10cm, 10-15cm and 15-20cm layer, respectively at Site A. Methane production (%) from hydrogenotrophic methanogenesis was 40 to 46% at 25°C, 37°C, and 45°C at the 0-10cm layer and 49% at 45°C in the 10-15cm layer. Acetate reduction was more pronounced at low temperatures until 15°C, dropped at 37°C and then increased at 45°C in the 0-10cm layer at Site A.

The dominant methanogenic pathway (%) varied with temperature but all over acetoclastic methanogenesis was dominant in all the three soil layers at Site B (Fig.30b). Acetoclastic methanogenesis dominated methane production from three soil layers of 0-10cm, 10-15cm and 15-20cm at Site B. The dominance of acetoclastic methanogenesis varied from 54 to 61%, 54 to 77% and 50 to 70% at different temperatures, in soil from the 0-10cm, 10-15cm and 15-20cm layer, respectively at Site B. Hydrogenotrophic methanogenesis was dominant only at 15°C (64%) and 37°C (70%) in the 0-10cm layer, at 45°C (63%) in the 10-15cm layer and 37°C (60%) in the 15-20cm layer.

At Site C, the production of methane from acetoclastic methanogenesis was dominant in the soil from 0-10 cm (73%) and bottom (56%) layer at 25°C. Methane was produced in equal amounts from hydrogenotrophic and acetoclastic methanogenesis in the soil from the 10-15cm at 25°C (Table 8).

In anaerobic environment where labile organic matter is always present in sufficient amounts, such as landfills and rice fields, the concentration of methanogenic precursors is likely to be constant. In wetlands environments, however the concentration of methanogenic precursors fluctuates enormously in time and varies significantly in space. At low temperatures either the substrate for hydrogenotrophic methanogens was unavailable (Schulz et al., 1997) or the low temperature reduced the activity of these group of methanogens (Conrad et al., 1987). The sediments of the White Oak River produced methane from both CO_2 reduction and acetate fermentation year round. The percentage of methane via acetate fermentation (70%)

and CO_2 reduction (30%) at this site is typical of freshwater environments where competition for substrates such as $SO4^{2-}$ is not an important process (Whiticar et al., 1986). In Buck Hollow peat, CO_2 reduction accounted for all the methane production in the wintertime and a portion of total methane production in the two springtime peat incubation experiments. However methane from acetate fermentation was also observed during May and June (Avery et al., 2002). In the small alasses of Siberia where the supply of fresh organic matter is abundant, methane was produced from equally from acetate fermentation and carbon dioxide reduction but in the larger alasses carbon dioxide reduction dominated slightly over acetoclastic methanogenesis owing to the smaller supply of labile organic matter (Nakagawa et al., 2002). In mineral sediments from lakes like Lake Wintergreen, acetoclastic methanogenesis accounts for 62% of methane production (Lovely et al., 1982) but in the sediments of Lake Lawrence, carbon dioxide reduction accounted for 86-97% of methane production. In acidic peat bog, hydrogenotrophic methanogenesis is the dominant pathway for methane production (Lansdown et al., 1992). The concentration of acetate is found to be very low at low pH, hence methane from acetoclastic methanogenesis is reduced in acidic peat soil. (Lansdown et al., 1992).

Peatlands are generally found to favour hydrogenotrophic methanogenesis but in Lake Khajjiar acetoclastic methanogenesis is more dominant. This may be explained by the low pH found in peatlands located especially in the temperate zone. Most known methanogens grow optimally at near-neutral pH (Zinder,1993; Boone et al., 1993). Methanogens can tolerate up to 100 mM volatile fatty acids at neutral pH; however, they are very sensitive to volatile fatty acids at mildly acidic pH (Berg et al., 1976; Wang et al., 1997). At lower pH such as 4.5, acetate and other volatile fatty acids are found to inhibit methanogenesis in bog peat but not at higher pH such as 6.5 (Horn et al., 2003). The pH of soil in lake Khajjiar is circumneutral and that could be the reason behind the dominance of acetoclastic methanogenesis.

In the mineral soils of Lake Bhalswa, the probable dominant pathway of methanogenesis is via acetate reduction at 25°C (Fig.50).

Methanogenic precursors

Accumulation of fatty acids was not observed in the soil slurries before incubation at different temperatures. Post incubation transient accumulation of acetate was dominant followed by propionate and butyrate. At Site-A and B, fatty acids did not accumulate in samples incubated over the wide range of temperature except at 45°C. Similar accumulation of fatty acids were also observed in sediments from Lake Constance when the soil was incubated above the optimum temperature (Schulz et al., 1997). However considerable accumulation of acetate, propionate and to a much lesser extent of butyarate was observed at higher temperatures, in the soil samples incubated with methyl fluoride at the 0-10cm at Site A. There was a slight accumulation of acetate in the 10-15 cm and 15-20cm soils incubated at higher temperatures at Site A (Fig.31a). The accumulation of acetate was observed over all temperatures at the 0-10cm and 10-15cm in the soils incubated with methyl fluoride at Site B (Fig.32a). The accumulation of acetate was not observed in soils incubated with methyl fluoride at 4°C and 37°C at Site B.

The accumulation of acetate and propionate was not equimolar as found earlier in mineral soils incubated with methyl fluoride. This could be because acetate was oxidized to CO_2 via aerobic respiration or other oxidative microbial processes (e.g., via the dissimilation of iron or nitrate) or oxidized syntrophically to CO_2 by the concerted activity of acetate-oxidizing anaerobes and hydrogenotrophic methanogens (Horn et al., 2003). The syntrophic degradation of acetate under anoxic conditions is found to occur in methanogenic bioreactors (Schnuerer et al., 1999) and lake sediments (Nuesslein et al., 2001). In soils incubated with methyl fluoride, a peak was observed which coeluted with butyrate but could not be identified.

External addition of methanogenic precursors stimulates the active group of methanogens and also gives an insight to the dominant pathway of methane production (Falz et al., 1999).

At 25°C, the stimulation of methane production by the addition of acetate and H_2/CO_2 varied across the soil depths and sites (Fig.45). H_2/CO_2 stimulated methane production more than acetate in soil from the 0-10 cm and 10-15cm at Site-1, and in

soil from the 15-20cm at Site-3. Acetate also stimulated methane production in the soils from 10-15cm of Site–3. No stimulation was observed in the other soil layers at the three sites. Methane production rates increased in the sediments of acidic bog when, supplemented with H_2/CO_2, ethanol, and 1-propanol, while formate, acetate, propionate, and butyrate inhibited the production of methane; methanol had no effect on it (Horn et al., 2003).

In the mineral soil of Lake Bhalswa significant stimulation of methane production by acetate was observed. H_2/CO_2 inhibited methane production (Fig.50).

Characterization of methanogens present in the soils of Lake Khajjiar

At Lake Khajjiar, the TRFLP analysis of soil layer from 0-10cm at Site-A and 0-10, 10-15, 15-20 cm at Site B of samples incubated with and without methyl fluoride (Fig.34-43) revealed 12 fragments of base pairs of which the four important ones were affiliated to *Methanobacteriaceae, Methanosarcinaceae*, *Methanosaetacaea* and *Methanomicrobiaceae* from the pre and post incubated soil. *Methanobacteriaceae* and *Methanomicrobiaceae* are known to be hydrogentrophs and *Methanosarcinaceae* and *Methanosaetaceae* are known to be acetoclastic methanogens. The other minor fragments of base pairs were affiliated to Crenarcheota Rice Cluster IV and VI, Euryarcheota -Rice Cluster I, II III and V. In the preincubated or the in-situ soil samples the relative abundance and composition of the base pair fragments did not differ significantly across the depth of soil cores. In the incubated samples the relative abundance and composition of the base pair fragments did not change with rise in temperature. A closer examination of the four major base pairs show that the relative abundance of the *Methanobacteriaceae* and *Methanomicrobiaceae* differed significantly across the three depths of soil core but no such change was observed in *Methanosarcinaceae* and *Methanosaetaceae.* Edwards et al., (1998) observed that sequences were distributed according to depth within the core from blanket bog at Moorhouse, UK. *Methanosarcinaceae* was detected at 8 to 30 cm depth, *Methanobacteriaceae* at soil depth from 16 to 30cm and *Methanosarcinaceae* and *Methanomicrobiaceae* were absent in any depth of the soil. The presence of all the four major groups of methanogens suggests that there is no shortage of substrate in

the soil at Lake Khajjiar. Though acetoclastic methanogenesis was more or less dominant in both the sites yet the abundance of the hydrogenotrophs (sum of fluorescence of base pair fragments affiliated to *Methanobacteriaceae* and *Methanomicrobiaceae*) and was more at all temperature across all the soil layer 0-10, 10-15 and 15-20 cm compared to the acetoclastic methanogens (*Methanosarcinaceae* and *Methanosaetaceae*).

Generally with the increase in temperature one would expect a shift in the relative gene frequency of *Methanosarcinaceae* and *Methanosaeataceae* (Wu et al., 2002). With the rise in temperature the availability of substrate increases, therefore *Methanosetacaea* that is relatively more active in low acetate concentration (as they are known to activate acetate with acetyl-CoA synthetase that is more efficient in scavenging low acetate concentrations than the acetate kinase in *Methanosarcina* species (Jetten *et al.*, 1990; 1992)), is replaced by the activity of *Methanosarcinaceae* that is known to thrive in high acetate concentration (Fey and Conrad, 2000; Chin *et al.*, 2004), Such a shift was not observed in case of Khajjiar soil. Similar observations were also found in the sediments of Lake Dagow. The change in the methanogenic pathway was not paralleled by a change of the community structure of the major phylogenetic groups (and guilds) of methanogenic archaea (Glissmann et al., 2004).

TRFLPs affiliated to *Methanomicrobiaceae, Methanobacteriaceae, Methanosarcinaceaea, Methanosaetaceae, Methanomicrobiaceae,* RC VI, RC III and RC IV were found to differ in the sediments at different sites of floodplain of River Waal (the Netherlands) as per the flooding regime (Kemnitz et al., 2004). *Methanomicrobiaceae, Methanobacteriaceae, Methanosarcinaceae* and the uncultured Rice Clusters IV and VI (*Crenarchaeota*) were detected in the frequently and permanently flooded soils. Crenarchaeotal sequences were the dominant group in the never and rarely flooded soils. *Methanosaetaceae*, were only found in the permanently and frequently flooded soils under conditions where concentrations of acetate were < 30mM.

Spatial difference was observed in the sequences detected from two wetlands Solvatnet and Stuphallet at Spitsbergen, of Norway (Arctic Wetlands). Though sequences affiliated with *Methanomicrobiales, Methanobacteriaceae, Methanosaeta*

and Group I.3b of the uncultured crenarchaeota were detected at both the wetlands, sequences affiliated with *Methanosarcina* were recovered only from the site Solvatnet, while sequences affiliated with the euryarchaeotal clusters Rice Cluster II and Sediment 1 were detected only at the site Stuphallet (Hoj et al., 2005).

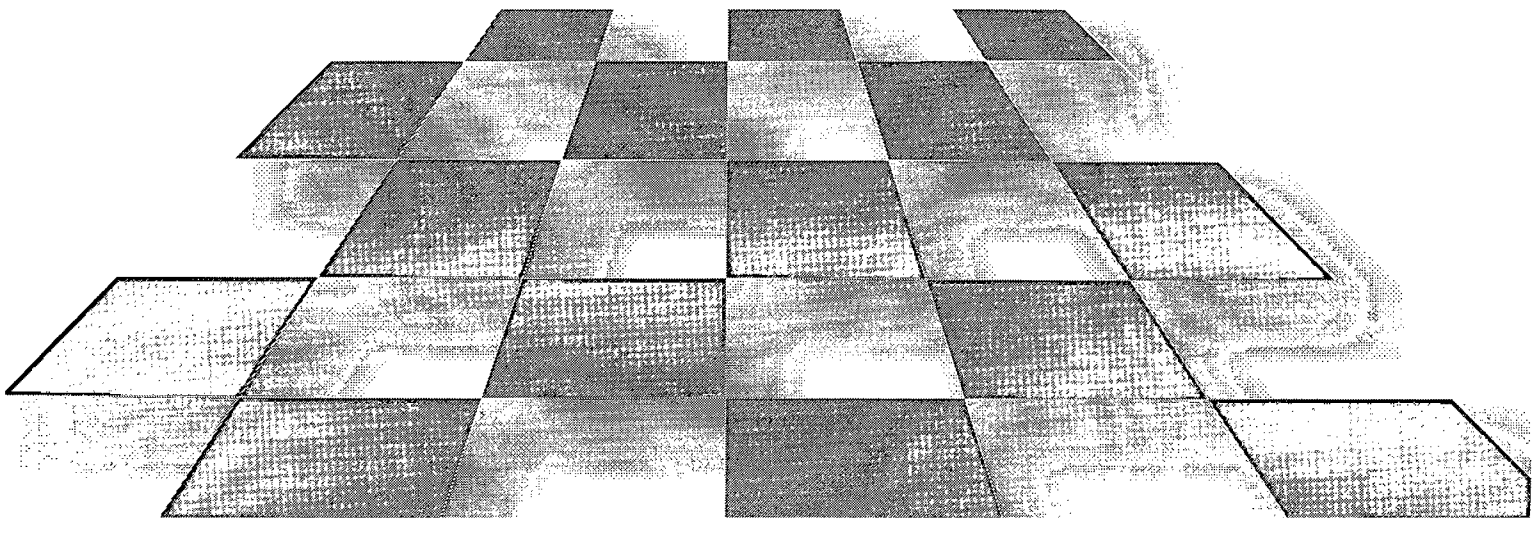
Peatlands across U.K and U.S.A showed similar methanogenic diversity. Upton et al. (2000) and McDonald et al., (1999) detected methanogenic groups *Methanosarcinaceae, Methanococcaceae, Methanobacteriaceae,* and *Methanomicrobiales* in peatlands of UK that were also observed in peat soils of Labrador Hollow and Mc Lean kettle bog with acidic pH by Basiliko et al (2003). Though some difference remained, *Methanosaetaceae* was observed in peatlands of U.S but not in peatlands of U.K.

The response of methanogens to the presence of alternate electron acceptors

The addition of nitrate (10mM) to the peat soils of Site 1, 2 and 3 at Lake Khajiar (collected in 2005) inhibited methane production as expected (Chidthaisong et al., 2000;Watson and Nedwell, 1998; Klueber and Conrad, 1998) but production of methane was not detected even after 45 days. Earlier incubation of rice field soil that contains much less labile organic substrate than peat soil recovered 30% methane production after 24 to 27 days of addition of nitrate (10mM). An addition of 20μM nitrate did inhibit the methane production but only for 43-57 days after which methane was detected (Klueber and Conrad, 1998). The immediate onset of the production of CH_4 in the headspace upon incubation indicated the absence of any other alternative electron acceptor in soils at Lake Khajjiar. Watson and Nedwell, (1998), found that even a 100μM of nitrate concentration did not inhibit methane production completely from the Ellergower peat soil. In the soils of Lake Khajjiar, nitrate was below the detection limit in both the method of analysis i.e, Ion Chromatographic method for porewater of the soils collected at 2004 and colorimetric method for the soils collected at 2005. This prolonged period of inhibition of methane production by the addition of 10μM nitrate in the nitrogen poor Khajjiar soil may be because the concentration of nitrogen increased beyond the immediate metabolism capacity of nitrate reducers, or production of toxic intermediates that inhibited methanogenesis (Scholten et al., 2002).

Methane production is inihibited by active sulfate reducing bacteria (Abram and Nedwell, 1978 a, b) when the concentration of sulfate increases in concentration (millimolar) (Lovely et al., 1982; Winfrey and Zeikus 1977). Reduced rate of methane production due to external addition of 10mM sodium sulfate was observed from all the layers of soil at Lake Khajjiar and Lake Bhalswa. In the soils of Site 1, 2 and 3 at Lake Khajjiar (collected in 2005) linear production of methane was inhibited for the first 15 days of incubation and then the rate increased but could never recover fully even after 36 days of incubation. The inhibition in the rate of methane production was significantly higher at Lake Khajjiar than at Lake Bhalswa. It was shown in batch cultures that sulfate reducers outcompete methanogens for H_2 due to a higher affinity and higher growth yield (Kristjansson et al., 1982, Robinson and Tiedje, 1984). Similarily, there are acetate degrading sulfate reducers who directly compete with methanogens for the available acetate (Scholten et al., 2002) and reduce the concentration of acetate below the uptake level of methanogens (Ingvorsen and Joergensen, 1984; Widdel, 1988). At Lake Khajjiar, 10μM of sodium sulfate inhibited methane production by nearly 90% or more at all the depth of soil from all the three sites (Fig. 44) whereas that very concentration of sulfate reduced methane production by 54% in the soil from Lake Bhalswa (Fig.49). Winfrey and Zeikus, (1977), observed that the addition of 0.2mM sulfate inhibited methane production for 10 hours, 10mM caused near complete inhibition for 100 hr (the recovery was only 4 to 5% of control) and intermediate concentrations of inhibited methanogenesis for a shorter period in soils from Lake Mendota. The extent of recovery of methane production on the inhibition of sulfate reducers by the external addition of molybdate was variable, reflecting the heterogeneity of the sediments. Nearly complete recovery of the rate of methane production was only observed from the 10-15cm soil layer of Site-3 (95.46 %) at Lake Khajjiar. The maximum recovery other than that was from the 0-10cm (54%) at Site-1 and the 0-10cm (44%) at Site-2. In the soil from Lake Bhalswa, the recovery in the rate of methane production was 92% after the inhibition of sulfate reducers on molybdate addition. Complete recovery of the rate of methane production was also observed in freshwater sediments of grasslands in Utrecht (Schloten et al., 2000). The incomplete recovery of methane production from soils of Lake Khajjiar suggests that there is other processes that are operational in inhibiting methane production on addition of sulfate either molybdate could not inhibit sulfate reducers completely.

CONCLUSIONS



Chapter VI
CONCLUSION

The study reveals the following:-

- At Lake Khajjiar, the rate of methane emission was found to vary significantly within the dominant plant species. Methane emitted by *Cyperus* sp. employ diffusion mechanism for the transport of methane and hence their capacity to emit methane is less compared to the *Acorus* and *Phragmites* sp. that posses the pressure-induced convective throughflow mechanism for the gas transport.

- *Scirpus* plants played a dominant role in methane flux from mineral soils. The dominance (%) of methane emission by *Scirpus* plants varied across the soil types and also months.

- Significant diel variation in the rate of methane emission was observed from the *Scirpus* plants grown in three types of soils (Bhalswa, Sandy and Garden). A significant peak that coincided with the sunrise was observed in the rate of methane emission from *Scirpus* plants irrespective of the soil type in which they were grown.

- The rate of emission from bare soils (Bhalswa, Sandy and Garden) did not vary significantly within the four months of study. The rate of methane emission from *Scirpus* plants grown in Garden soil was significantly higher than Bhalswa soil and Sandy soil.

- Weak correlation between the biomass and total methane emission was found in the *Scirpus* grown in Bhalswa Soil. A significant correlation is observed between the total methane emission and biomass of *Scirpus* plants grown in Sandy soil and Garden soil.

- The rate of methane emission from deep submerged *Scirpus* plants (30 cm) was significantly higher than the shallow submerged *Scirpus* sp.Methane

Crenarcheota Rice Cluster IV and VI, Euryarcheota -Rice Cluster I, II, III and V. *Methanobacteriaceae* and *Methanomicrobiaceae* are known to be hydrogenotrophs that use H_2/CO_2 as substrate and *Methanosarcinaceae* and *Methanosaetaceae* are known to be acetoclastic methanogens that use basically acetate as substrates. The sum of T-RF's affiliated to *Methanobacteriaceae* and M*ethanomicrobiaceae* was greater than the sum of *Methanosarcinaceae* and *Methanosaetaceae* across all temperatures in different soil layer at sites A and B.

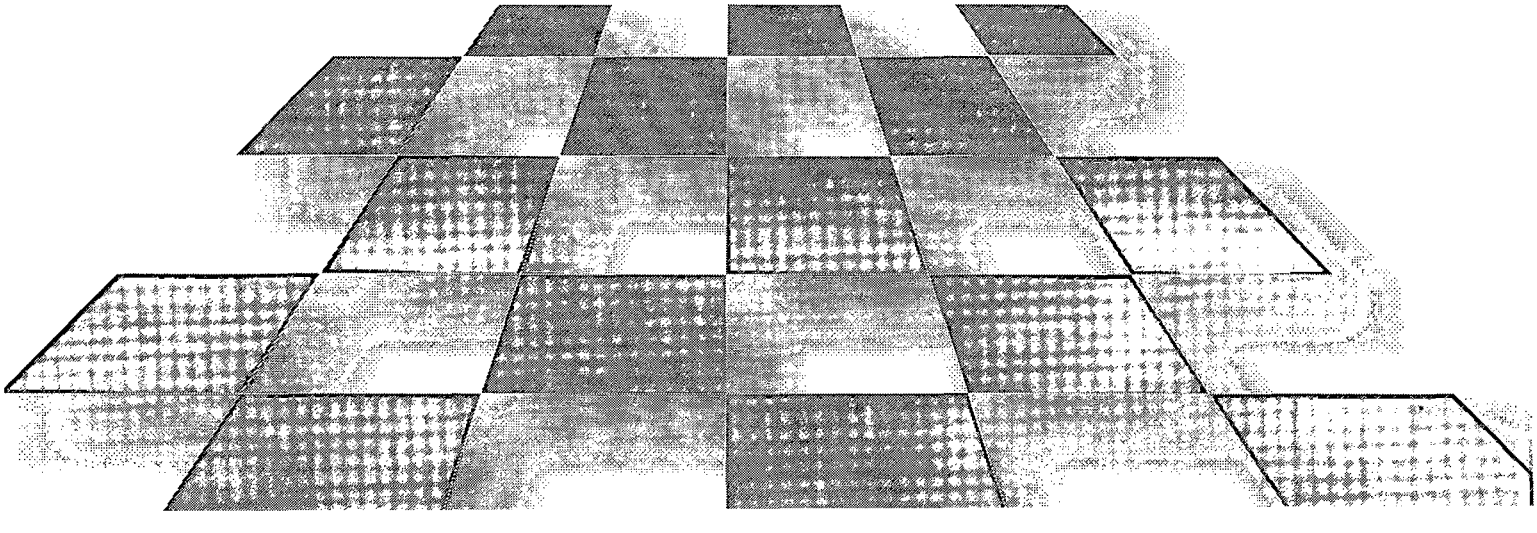
- At 25°C, the stimulation of methane production on the addition of acetate and H_2/CO_2 varied across the soil depth and site. In the mineral soil of Lake Bhalswa significant stimulation of methane production from acetate was observed. H_2/CO_2 inhibited methane production.

- Addition of nitrate (10mM) to the peat soils of Lake Khajiar inhibited methane production but methane was not detected even after 45 days.

Reduced rate of methane production due to external addition of 10mM sodium sulfate was observed from all the layers of soil in Lake Khajjiar and Lake Bhalswa. In the soils of Lake Khajjiar production of methane was inhibited for the first 15 days of incubation and then the rate increased but could never recover fully even after 36 days of incubation.

The inhibition in the rate of methane production by sulfate was significantly higher in Lake Khajjiar than in Lake Bhalswa. The extent of recovery of methane production on the inhibition of sulfate reducers by the external addition of molybdate was variable, reflecting the heterogeneity of the sediments.

SUMMARY



Chapter VII

SUMMARY

Methane is a greenhouse gas and 23 times more potent in global warming compared to carbon-dioxide. Wetlands by the virtue of their waterlogging provide a suitable habitat for the methanogens. Wetland plants act as conduits to the methane produced in the deep rhizospheric zone by the methanogens. Hence, the emission of methane is a resultant of methane production and oxidation. Plants employ two different mechanism for gas transport:- molecular diffusion a passive process where gases diffuse due to concentration gradient and pressure-induced convective throughflow where the pore spaces in the plant tissue is below the Knudsen regime (0.1μm) and the transport is propelled by the temperature or humidity difference between the external and internal atmosphere of plant tissues. Plants do not only play a passive role but its exudates from the roots and addition of organic matter due to root and leaf sloughing makes it an active factor in the regulation of methane emission. Other than that the transport of oxygen to the rhizosphere promotes methanotrophs and inhibits the activity of methanogens.

Methanogens, belonging to the kingdom Archaea, produce methane only in an anoxic environment. Methanogens generally use acetate or H_2/CO_2 and produce methane. Acetate, H_2 and CO_2 are products of the microbial degradation of organic matter under anoxic conditions

The production, oxidation and emission of methane from wetlands form a major priority in research around the world. In India, the major focus has been on rice fields and wetlands were hardly studied.

Two different natural wetlands in India were studied. The selection of the natural wetlands was based on edaphic factors- mineral and organic soil. The study was aimed to achieve the following objectives.

As mentioned before, the presence of plants alters the contribution of methane produced in the soil to the atmosphere. Species of plants also differ in their capacity

of methane emission; hence it was aimed to find the role of plants in methane emission present in the two different natural wetlands.

The same species of plant differ in their capacity of methane emission in different soil types. The plants also differ in their rate of emission with the different stages of growth. Depth of water table influences the production of methane as the anoxicity level varies with the fluctuation. The influence of the height and pressure of the overlying water on the rate of emission has been studied poorly. The aim of the study was to study the influence of soil types, plant growth and water-depth on methane emission from *Scirpus* sp. in a mineral wetland.

The rate of methane production changed with the change in temperature and the optimum temperature for maximum methane production varied with soil types. Thus, the objective was set to determine the rate of methane production from the soils at different temperatures.

Methanogens produce methane by utilizing either H_2/CO_2 or acetate. The study was aimed to characterize the different groups of methanogens on the basis of their substrate utilization in the two natural wetland soils.

The presence of external electron acceptors such as nitrate or sulfate that are seated higher in the electron reduction series inhibits the activity of methane production to varying extent. Hence, the study was aimed to assess the response of the methanogens to the presence of different electron acceptors in the soils of in the two different wetlands.

Lake Khajjiar in Himachal Pradesh is one of the few pockets in upper Himalayas where one can find peat soil. The soil from this wetland was sampled twice-once in 2004, June and the second time in August, 2005. The soil collected from the three sites at the pheriphery of the Lake during the first sampling was subjected to study at Max Planck Institute for Terrestrial Microbiology, Marburg. During sampling 0-10cm, 10-15cm and 15-20cm sections of soil core were dug from the three sites. An initial experiment referred as pilot experiment was conducted to assess the production and

oxidation capacity of soils from Site C, at 25°C. Later the experiments were focused on methane production, though the soil was found to be potent in methane production and oxidation as well. The soils from different layers at Site-A and B, was incubated in an anoxic environment at six different temperatures and the methanogenic community was determined before and after incubation at different temperatures by using molecular techniques. The soils from different layers at Site B and C were incubated with methyl fluoride at the above-mentioned temperatures to gain an understanding of the probable pathway of methane production. Methanogenic community from soils incubated with methyl fluoride at Site B was also analysed. The rate of production of carbon dioxide was also determined along with methane. Fatty acids were analysed from pore water from soils with or without methyl fluoride pre and post incubation at different temperatures.

In August 2005, methane emission from the dominant vegetation and bare soil at three different sites around the pheriphery of the Lake was sampled. Methane emission from *Acorus* and *Phragmites* sp. At Site 1, *Acorus* and *Cyperus* sp. at Site 2 and *Phragmites* sp. at Site 3 was sampled. Soil samples of 0-5cm, 5-15cm and 15-25cm depth were dug out from the above sites. These soils of different layers at Site 1, 2 and 3 were incubated with external addition of sodium nitrate (10mM), sodium sulfate (10mM) and sodium molybdate (10mM) (an inhibitor used for sulfate reducers). The soils from different layers were also amended with substrates like sodium acetate and H_2/CO_2. The stimulation of methanogens with the addition of external substrates reveals the probable pathway of methane production from these soils. The different soil types were incubated in an anoxic environment at four different temperatures (11, 25, 37 and 48°C) to determine the optimum temperature at which there is maximum methane production.

Soils from Lake Bhalswa were collected and transported to the Garden of Environmental sciences where a detailed study with *Scirpus* plants grown in these soils was undertaken. Apart from Bhalswa Soil, terrestrial soil mixed with sand referred as Sandy Soil and terrestrial soil mixed with organic manure referred as Garden soil was used to study the effect of soil types on methane emission from *Scirpus* plants. The rate of methane emission from *Scirpus* plants grown in Bhalswa

soil and Sandy soil were sampled in May, June, July and October. The rate of methane emission from *Scirpus* plants grown in Garden soil was sampled in June, July and October.

The soils from Lake Bhalswa were also incubated with sodium sulfate and sodium molybdate. Soils from Lake Bhalswa were amended with substrates such as sodium acetate and H_2/CO_2 to know the dominant pathway of methane production.
Scirpus plants grown on same soil type (garden soil) were subjected to two different water depth (+15cm) and (+30cm) and dryness with water table 2-3cm below the soil surface. The oxidation capacity of the shoots (36 cm shoot length from base), roots and rhizomes, primary roots and rhizospheric soil was assessed for *Scirpus* plants growing on the soil growing at waterlogged soil.

The study reveals the following: -

The role of plants in methane emission

At Lake Khajjiar, the rate of methane emission varied significantly within the dominant plant species. Methane emitted by *Cyperus* sp. employ diffusion mechanism for the transport of gases and hence their capacity to emit methane is less compared to the *Acorus* and *Phragmites* sp. that posses the pressure-induced convective throughflow mechanism for the gas transport.

Scirpus plants played a dominant role in methane flux from mineral soils. The dominance (%) of methane emission by *Scirpus* plants varied across the soil types and also months. With the exception of occasional upsurge (72%) of soil efflux from bare Garden soil in October, methane efflux did not exceed the flux from the vegetated soil. Significant diel variation in the rate of methane emission was observed from the *Scirpus* plants grown in three types of soils (Bhalswa, Sandy and Garden). A significant peak that coincided with the sunrise was observed in the rate of methane emission from *Scirpus* plants irrespective of the soil type in which they were grown suggesting the role of stomata in venting gases.

The rate of emission from bare soils (Bhalswa, Sandy and Garden) did not vary significantly within the four months whereas the rate of methane emission from the *Scirpus* plants grown in Sandy and Garden soil varied significantly over the months.

Influence of edaphic and hydrological conditions on methane emission

The rate of methane emission from *Scirpus* plants grown in Garden soil was significantly higher than in Bhalswa soil and Sandy soil. The reason for the stable rate of methane emission over the four months from the *Scirpus* plants grown in Bhalswa soils may be due to the high clay content in these soils that ensured a regular supply of substrates by mineralization and a gradual release of gas that may be entrapped by the clay. A strong correlation was observed between the organic matter content and the total methane emission from the *Scirpus* plants in Bhalswa soil (r^2=0.944).

Rate of methane emission from senescing *Scirpus* plants grown in Garden and Sandy soil decreased in October. A significant correlation is observed between the total methane emission and biomass of *Scirpus* plants grown in Sandy soil and Garden soil. Weak correlation between the biomass and total methane emission was found in the *Scirpus* plants grown in Bhalswa Soil.

The rate of methane emission from deep submerged *Scirpus* plants (30 cm) was significantly higher than the shallow submerged *Scirpus* sp. The concentration of methane emission from *Scirpus* plants, which grew on soils with the water table below the soil surface, was not above the atmospheric concentration of methane. The lowering of water table could be related to situations when wetlands dry up temporarily, exposing the soil to oxygen. Methane consumption was observed in all the plant parts as well as rhizospheric soil. The maximum methane consumption was observed in primary roots followed by roots and rhizomes, shoots (till 36 cm plant length from the base) and then rhizosperic soil. Methane consumption by the shoots of *Scirpus* sp. indicates that substantial quantity of methane that is released from the plants is consumed at the surface. This could also explain the higher rate of methane emission from *Scirpus* sp.that were inundated to a higher depth compared to those that were less inundated.

Influence of temperature on methane production

The optimum temperature for maximum methane production from the soil layers at Site A and B of Lake Khajjiar was 37°C. This optimum temperature is far higher than the in-situ temperature experienced at any time of the year (annual average range ~2 and 25.5°C). The optimum temperature of maximum rate of methane production was 48°C in the mineral soils (Lake Bhalswa, Sandy and Garden soil). The optimum temperature for maximum methane production at 48°C was higher in the soils at Lake Bhalswa (mineral soil) compared to 37°C at Lake Khajjiar (peat soil). In the soil cores 0-10, 10-15 and 15-25cm at Lake Khajjiar, the rate of methane production generally reduced with the depth of soil core.

Rate of methane production was determined from soil samples at different sites at Lake Khajjiar in 2004 as well as 2005. The rate of methane production was two-fold higher in the soil samples collected in August 2005 than the soils collected in June, 2004. This may be due to the difference in season as June is summer times and August is rainy season when the availability of organic matter is more due to runoff. This fact is also revealed in the difference in the organic matter content between two sampling. Rate of methane production was higher from the peat soils at Lake Khajjiar compared to the mineral soil at Lake Bhalswa.

The rate of organic carbon mineralization (determined from the rate of production of carbon-dioxide and methane) at lake Khajjiar increased with the rise in temperature but the values were low compared to the values reported from other wetlands.

Accumulation of fatty acids was not observed in the soil slurries before incubation at different temperatures. Post incubation transient accumulation of acetate was dominant followed by propionate and butyrate. At Site-A and B, fatty acids did not accumulate in samples incubated over the wide range of temperature except at 45°C. Considerable accumulation of acetate and propionate to a much lesser extent of butyarate was observed at higher temperatures, in the soil samples incubated with methyl fluoride at the 0-10cm at Site A. There was a slight accumulation of acetate in the middle and 15-20cms at samples incubated at higher temperatures at Site A. The accumulation of acetate was observed over all temperatures at the top and 10-15cm

from the soil samples incubated with methyl fluoride at Site B. In the soil samples incubated with methyl fluoride at 15-20cm at Site B no accumulation of acetate was observed at 4° and 37°C.

The accumulation of acetate and propionate was not equimolar in soils at Lake Khajjiar as found earlier in mineral soils incubated with methyl fluoride. The above two findings of RI and fatty acids indicate that processes (could not be specified) other than methanogenesis are also operational in this system.

Identification of dominant pathways of methane production in two natural wetlands

Peatlands are generally found to favour hydrogenotrophic methanogenesis but in Lake Khajjiar, acetoclastic methanogenesis is dominant. Acetoclastic methanogenesis was also dominant in the soils of Lake Bhalswa. The dominance of acetoclastic methanogenesis varied with temperature and depth of soil core (0-10, 10-15 and 15-20cm) at the two different sites of Lake Khajjiar. The dominance ranged from 50-90% in different soil layers across different temperatures at Site A of Lake Khajjiar. At Site–B methane produced by hydrogenotrophic methanogenesis was dominant at 15°C (64%) and 37°C (70%) in the 0-10cm layer, at 45°C (63%) in the 10-15cm layer and 37°C (60%) in the 15-20cm layer. Apart from the above mentioned soil layers different temperatures, methane production from acetoclastic methanogenesis was found to be dominant in the rest of the soil layers.

At Lake Khajjiar, the TRFLP analysis revealed 12 fragments of base pairs of which the four important ones were affiliated to *Methanobacteriaceae, Methanosarcinaceae*, *Methanosaetacaea* and *Methanomicrobiaceae* from the pre and post incubated soil. The other minor ones were affiliated to Crenarcheota Rice Cluster IV and VI, Euryarcheota -Rice Cluster I, II III and V. *Methanobacteriaceae* and *Methanomicrobiaceae* are known to be hydrogenotrophs that use H_2/CO_2 as substrate and *Methanosarcinaceae* and *Methanosaetaceae* are known to be acetoclastic methanogens that use basically acetate as substrates. The sum of T-RF's affiliated to *Methanobacteriaceaea* and *Methanomicrobiaceae* was greater than the sum of *Methanosarcinaceae* and *Methanosaetaceae* across all temperatures in different soil layer at site A and B. The relative abundance of the *Methanobacteriaceae* and

Methanomicrobiaceae differed significantly across the three depths of soil core but no such change was observed in *Methanosarcinaceae* and *Methanosaetaceae.*

At 25°C, the stimulation of methane production on the addition of acetate and H_2/CO_2 varied across the soil layers (0-10, 10-15 and 15-20 cm) and three sites at Lake Khajjiar whereas in the mineral soil of Lake Bhalswa significant stimulation of methane production from acetate was observed. H_2/CO_2 inhibited methane production in soils from Lake Bhalswa.

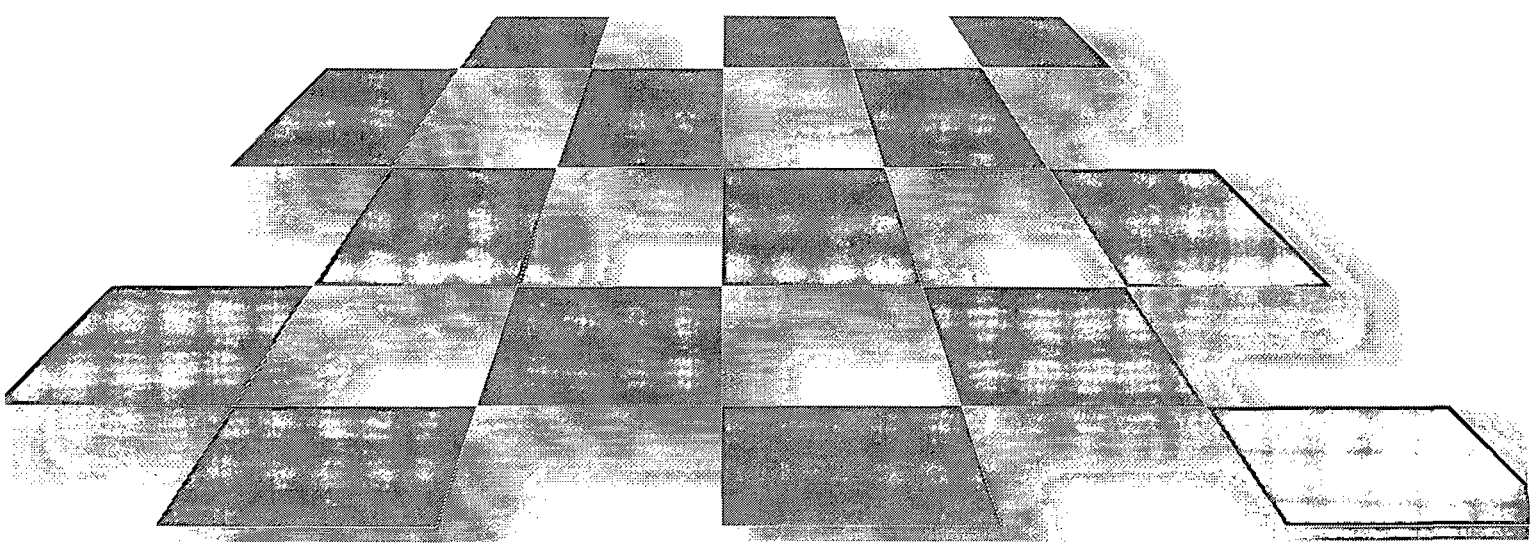
The response of methanogens in the presence of alternate electron acceptors

Addition of nitrate (10mM) to the peat soils of Lake Khajjiar inhibited methane production and production of methane was not detected even after 45 days. Reduced rate of methane production due to external addition of 10mM sodium sulfate was observed from all the layers of soil at Lake Khajjiar and from soil at Lake Bhalswa. In the soils layers at Lake Khajjiar linear production of methane was inhibited for the first 15 days of incubation and then the rate increased but could never recover fully even after 36 days of incubation. The inhibition in the rate of methane production by sulfate was significantly higher in soil layers at different sites at Lake Khajjiar than in soils at Lake Bhalswa.

In conclusion, the above study presents the determination and estimation of methane production and emission from two natural wetlands, Lake Khajjiar and Lake Bhalswa. Plants do play a major role in emitting gases as found from *Scirpus* plants grown in different mineral soils but exceptions are reflected in methane emission from the dominant plants at the two Sites (1 and 2) in Lake Khajjiar where these plants emit less methane compared to the bare soil. Soil types differing in texture influence methane emission, as evident from the different rate of methane emission from *Scirpus* plants growing in the Bhlaswa, Sandy and Garden soil. Different stages of plant growth do influence methane emission but the influence varied from one soil type to another. The changes in the water level and water table do influence the rate of methane emission. Rate of methane emission reduced with the increase in water level. Oxidation of methane was highest in the primary roots followed, roots and rhizomes shoot parts and by rhizosphere.

Rate of methane production varied between the two wetlands due to the difference in soil types; the soil of Lake Khajjiar is peaty and the soil from Lake Bhalswa is of mineral type, but the dominant pathway of methanogenesis was same. The methanogenic community at Lake Khajjiar was more sensitive to the external addition of sulfate and molybdate compared to Lake Bhalswa.

REFRENCES



Chapter VIII

REFERENCES

Abram, J.W & Nedwell, D.B.1978a. Inhibition of methanogenesis by sulphate reducing bacteria competing for transferred hydrogen. *Archives of Microbiology.* 117(1):89-92.

Abram, J.W & Nedwell, D.B.1978b. Hydrogen as a substrate for methanogenesis and sulphate reduction in anaerobic saltmarsh sediment. *Archives of Microbiology.* 117(1): 93-97

Achtnich, C.F. & Rude, P.D. 1988. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. *Biol. Fertil. Soils.*19: 65–72.

Achtnich, F. Bak & R. Conrad. 1995. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil, *Biol. Fertil. Soils.* 19: pp. 65–72.

Adhya, T.K., Bharati, K., Mohanty, S.R., Ramakrishnan, B., Rao, V.R., Sethunathan, N.& Wassmann, R.2000. Methane emission from rice fields at Cuttack, India. *Nutrient Cycling in Agroecosytems.* 58: 95-105.

Allen, Jr L. H., Albrecht, S. L., Wilfredo Colo´ n-Guasp, Covell, S. A., Baker, J. T., Deyun Pan, & Boote, K. J. 2003.Methane Emissions of Rice Increased by Elevated Carbon Dioxide and Temperature. *J. Environ. Qual.* 32:1978–1991 .

Allen, S. D., & Brock, T. D. 1968. The adaptation of heterotrophic microcosms to different temperatures. *Ecology* 49:343–346.

Alm, J., Saarnio, S., Nyka¨nen, H., Silvola, J. & Martikainen, P.J., 1999. Winter CH_4, CO_2 and N_2O fluxes on some natural and drained boreal peatlands. *Biogeochemistry* 44:163–186.

Altor, A. E. and Mitsch, W.J. 2006. Methane flux from created wetlands: Relationship to intermittent vs. continuous inundation and emergent macrophytes. *Ecological Engineering.* 28: 224-234.

Anderson, J.M. & Ingram, J.S.I.1989. Tropical soil biology and fertility: A handbook of methods. C.A.B International. U.K.

Andrews, J.A., Matamala, R., Westover, K.M., Schlesinger, W.H. 2000. Temperature effects on the diversity of soil heterotrophs and the 13C of soil-respired CO_2. *Soil Biol Biochem.* 32:699–706

Anthony, C. (1982). The biochemistry of Methylotrophs. Academic Press, London.

Anthony, C. (1986). Bacterial oxidation of methane and methanol. Advances in Microbial Physiology.27:113-210.

Armstrong, J. & Armstrong, W. 1991. A convective throughflow of gases in *Phragmites australis* (Cav.) Trin. Ex. Steud. *Aquatic Botany.* 39: 75–88.

Armstrong, W., Armstrong, J., Beckett, P.M & Justin, S.H.F.W. 1991a. Convective gas-flows in wetland plant aeration. In: M.B. Jackson, D.D. Davies and H. Lambers (Editors), Plant Life Under Oxygen Stress. SPB Academic Publishing B.V., The Hague, The Netherlands, 283-302.

Armstrong, W.1979. Aeration in higher plants. In: H.W.W. Woolhouse (Editor), Advances in Botanical Research. Vol. 7, Academic Press, London.pp. 225-332.

Avery, G. B., R. D. Shannon, J. R. White, C. S. Martens, and M. J. Alperin. 2002. Controls on methane production in a tidal freshwater estuary and a peatland:

Methane production via acetate fermentation and CO2 reduction. *Biogeochemistry* 62:19-37.

Avery, G.B. Jr & Martens, C.S. 1999b. Controls on the stable isotopic composition of methane produced in a tidal freshwater estuarine sediment. *Geochim. Cosmochim. Acta.* 63: 1075–1082.

Avery, G.B. Jr, Shannon, R.D., White, J.R., Martens, C.S. & Alperin M.J. 1999a. Effect of seasonal changes in the pathways of methane production on the _13C values of pore water methane in a Michigan peatland. *Global Biogeochem. Cycles* 13: 475–484.

Avery, G.B., Shannon, R.D., White, J.R., Martens, C.S. & Alperin, M.J. 2003. Controls on methane production in a tidal freshwater estuary and a peatland: methane production via acetate fermentation and CO2 reduction. *Biogeochemistry.*62: 19–37.

Balderston, W.L. & Payne, W.J. 1976 Inhibition ofmethanogenesis in salt marsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen oxides, *Appl. Env. Microbiol.* 32: 264-269.

Banker, B. C., Kludze, H. K., Alford, D. P., DeLaune, R. D & Lindau, C. W. 1995. CH_4 sources and sinks in paddy rice soils, relationship to emissions. *Agric. Ecosys. Environ.* 53: 243-225.

Bartlett, K.B. & Harriss, R.C. 1993. Review and assessment of methane emissions from Wetlands.*Chemosphere* 26: 261–320.

Bartlett, K.B., Crill, P.M., Sebacher, D.I., Harriss, R.C.,Wilson, J.O., & Melack, J.M. 1988. Methane flux from the central Amazonian floodplain. *J Geophys Res* 93: 1571–1582.

Bartlett, K.B, Bartlett, D.S., Harriss, R.C. & Sebacher, D.I. 1987.Methane emissions along a salt marsh salinity gradient. *Biogeochemistry* 4: 183–202.

Basiliko, N. & Yavitt, J.B. Influence of Ni, Co, Fe, and Na additions on methane production in *Sphagnum*-dominated Northern American peatlands. *Biogeochemistry* **52:** 133–153, 2001.

Basiliko, N., Yavitt, J. B., Dees, P. M., & Merkel, S. M. 2003. Methane biogeochemistry and methanogen communities in two northern peatland ecosystems, New York State. *Geomicrobiol. J.* 20:563–577.

Bellisario, L.M., Bubier, J.L., Moore, T.R. & Chanton, J.P. 1999. Controls on CH_4 emissions from a northern peatland. *Global Biogeochemical Cycles* 13: 81–91.

Bender M & Conrad R (1993) Kinetics of CH4 oxidation in oxic soils, *Chemosphere.* 26: 687-26696

Bender M & Conrad R (1994) Methane oxidation activity in various soils and freshwater sediment: Occurrence, characteristics, vertical profiles, and distribution on grain size fractions. *J Geophys.Res* 99(48): 16531–16540

Bendix, M., Tornbjerg, T. & Brix, H. 1994. Internal gas transport in Typha latifolia, L. and Typha angustifolia, L.I Humidity-induced pressurization and convective throughflow. *Aquatic Botany.*49:75-90.

Berg, L van den., Patel, G. B., Clark, D. S. and Lentz, C. P. 1976. Factors affecting rate of methane formation from acetic acid by enriched methanogenic cultures. *Can. J. Microbiol.* 22:1312-1319.

Berg, B., McClaugherty, C. & Johansson, M.B. 1993. Litter mass-loss rates in late stages of decomposition at some climatically and nutritionally different pine sites. Long-term decomposition in a Scots pine forest. VIII. *Canadian Journal of Botany.* 71: 680–692.

Blodau, C.& Moore, T.R., 2003. Micro-scale CO_2 and CH_4 dynamics in a peat soil during a water fluctuation and sulfate pulse. *Soil Biology and Biochemistry.* 35: 1–13.

Bodegom P.M. van & Stams A.J.M. 1999. Effects of alternate electron acceptors and temperature on methanogenesis in rice soils. *Chemosphere* 39(1): 167–182.

Boeckx, P. & Cleemput, O. 1996. Methane emission from a landfill and the methane oxidizing capacity of its covering soil. *Soil Biology and Biochemistry.*28:1397-1405.

Boeckx, P. and Van Cleemput, O. 1997, Methane emissions from a freshwater wetland in Belgium. *Soil Sci. Soc. Am. J.* 61, 1250–1256

Bonkowski, M., Cheng, W., Griffiths, B.S., Alphei, J. & Scheu, S. 2000. Microbial–faunal interactions in the rhizosphere and effects on plant growth. *European Journal of Soil Biology* **36**: 135–147.

Boon, P.I & Mitchell, A. 1995. Methanogenesis in the sediments of an Australian freshwater wetland: Comparison with aerobic decay, and factors controlling methanogenesis. *FEMS Microbiology Ecology.* 18: 175- 190.

Boon, P.I., Mitchell, A., and Lee, K. 1997. Effects of wetting and drying on methane emissions from ephemeral floodplain wetlands in south-eastern Australia. *Hydrobiologia* 357: 73–87.

Boone, D. R. & Whitman, W. B. 1988. Proposal of minimal standards for describing new taxa of methanogenic bacteria. *Int J Syst Bacteriol* 38: 212±219.

Boone, D.R., Whitman, W.B. & Rouviere, P. 1993. Diversity and taxonomy of methanogens. In Ferry J.G. (ed), Methanogenesis, pp. 35±80. Chapman and Hall Co., New York. Academic Press, Orlando

Born, M., Doerr, H. & Levin, I. 1990. Methane consumption in aerated soils of the temperate zone. *Tellus . 42B:* 2-8.

Bosse, U.& Frenzel, P. 1997. Activity and distribution of methane-oxidizing bacteria in flooded rice soil microcosms and in rice plants (oryza sativa). *Appl. Environ. Microbiol.*63 : 1199–1207.

Bremner, J.B. 1965. Methods of soil analysis. Part 2. Chemical and microbiological Properties.Edited by Black,C.A.Evans,D.A., Ensminger,L.E., White,J.L., Clark,F.E.,Dinauer,R.C. American Soc. of Agronomy, Inc., Publisher. USA.

Brinson, M.M. 1993a. Changes in the functioning of wetlands along environmental gradients. Wetlands.13:65-74.

Brinson, M.M. 1993b. Ahydrogeomorphic classification for welands. Technical Report WRP-DE-4. US Army Corps of Engineers,Washington, DC.

Brix, H. Sorrell, B.K. & Orr, P.T. 1992. Internal pressurization and convective gas flow in some emergent freshwater macrophytes. *Limnol. Oceanogr.* 37(7): 1420-1433.

Brix, H. 1990. Gas exchange through soil-atmosphere interface and through dead culms of *Phragmites australis* in a constructed reed bed receiving domestic sewage.*Water Res*:24, 259-266.

Bubier, J. l. and Moore, T.R. 1994. An ecological perspective on methane emissions from northern wetlands. *Trends Ecol. Evol.*9:460-464.

Bubier, J., Crill, P., Mosedale, A., Frolking, S. & Linder, E. 2003b. Peatland responses to varying interannual moisture conditions as measured by automatic CO2 chambers. *Global Biogeochemical Cycles.* 17, 1066.

Bubier, J.L. 1995. The relationship of vegetation to methane emission and hydrochemical gradients in northern peatlands. *J. Ecology* 83: 403–420.

Bubier, J.L., Bhatia, G., Moore, T.R., Roulet, N.T. & Lafleur, P.M. 2003a. Spatial and temporal variability in growing-season net ecosystem carbon dioxide exchange at a large peatland in Ontario, Canada. *Ecosystems* 6, 353–367.

Bubier, J.L., Moore, T.R. & Roulet, N.T. 1993. Methane emissions from wetlands in the midboreal region of northern Ontario, Canada. *Ecology* 74: 2240–2254.

Burdige D.J. 1993. The biogeochemistry of manganese and iron reduction in marine sediments. *Earth-Science Reviews* 35: 249–284

Cadillo-Quiroz, H, Brouer, S.,Yashiro, E., Sun, C., Yavitt, J.B. & Zinder,S. 2006. Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York. Environmental Microbiology. 8 (8), 1428–1440 .

Capone D.G. & Kiene R.P. 1988. Comparison of microbial dynamics in marine and freshwater sediments: contrast in anaerobic carbon catabolism. *Limnology and Oceanography.* 33: 725–749.

Chan, C.C., Claus, P., Casper, P., Ulrich, A., Leuders, T. & Conrad, R. 2005.Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. Environmental Microbiology 7(8): 1139–1149

Chanton J. P., Bauer J. E., Glaser P. A., Siegel D. I., Kelley C. A., Tyler S. C., Romanowicz E. H., & Lazrus A. 1995. Radiocarbon evidence for the substrates supporting methane formation within northern Minnesota peatlands. *Geochim. Cosmochim. Acta* 59(17): 3663–3668.

Chanton, J.P. & Dacey, J.W.H. 1991. Effects of vegetation on methane flux reservoirs andcarbon isotopic composition. In: Sharkey, T., Holland, E., Mooney, H. (Eds.), Trace Gas Emissions From Plants. Academic Press, San Diego, pp. 65–92

Chanton, J.P. & Whiting, G.J. 1996. Methane Stable Isotopic distributions as indicators of gas transport mechanisms in emergent aquatic plants. *Aquatic Botany.* 54: 227–236.

Chanton, J.P., Arkebauer, T.J., Harden, H. & Verma, S.B. 2002. Diel variation in lacunal CH_4 and CO_2 concentration and d13C in *Phragmites australis*. *Biogeochemistry* 59: 287–301.

Chanton, J.P., Chasar, L.C., Glaser, P. & Siegel, D. 2005. Chapter 6, Carbon and hydrogen isotopic effects in microbial methane from terrestrial environments. In: Flanagan, J.R., Ehleringer, J.R., Pataki, D.E. (Eds.), Stable Isotopes and Biosphere–Atmosphere Interactions, Physiological Ecology Series. Elsevier–Academic Press, pp. 55–105.

Chanton, J.P., Martens, C.S., Kelley, C.A., Crill, P.M. & Showers, W.J. 1992a. Methane transport mechanisms and isotopic fractionation in emergent macrophytes of Alaskan tundra lakes. *Journal of Geophysical Research.* 97: 681–688.

Chanton, J.P., Whiting, G.J., Blair, N.E., Lindau, C.W. & Bollich, P.K. 1997. Methane Emission From Rice: stable isotopes, diurnal variations and CO2 exchange. *Global Biogeochemical Cycles.* 11: 15–27.

Chanton, J.P., Whiting, G.J., Happel, J.D. & Gerard, G.1993. Contrasting rates and diurnal patterns of methane emission from emergent aquatic macrophytes. *Aquatic Botany.* 46: 111-128.

Chanton, J.P., Whiting, G.J., Showers, W.J. & Crill, P. 1992b. Methane flux from *Peltandra virginica* - stable isotope tracing and chamber effects. *Global Biogeochemical Cycles.* 6, 15–33.

Chidthaisong, A. & Conrad, R. 2000. Turnover of glucose and acetate coupled to reduction of nitrate, ferric iron and sulfate and to methanogenesis in anoxic rice field soil. *FEMS Microbiol Ecol* 31: 73–86.

Chin, K.J., and Conrad, R. 1995. Intermediary metabolism in methanogenic paddy soil and the influence of temperature. *FEMS Microbiol. Ecol.* 18:85–102

Chin, K.J., Lueders, T., Friedrich, M. W., Klose, M., and Conrad, R. 2004. Archaeal community structure and pathway of methane formation on rice roots. *Microb. Ecol.* 47:59–67.

Chin, K.J., Lukow, T. & Conrad, R. 1999. Effect of temperature on structure and function of the methanogenic archaeal community in an anoxic rice field soil. *Appl. Environ. Microbiol.* 65 : 2341–2349.

Chow, A. T., Tanjia, K.K., Gaob, S. & Dahlgrena, R. A. 2006. Temperature, water content and wet–dry cycle effects on DOC production and carbon mineralization in agricultural peat soils. 2006. *Soil Biology & Biochemistry* 38: 477–488.

Christensen, T.R., Jonasson, S., Callaghan,T.V. & Havstroem,M. 1999. On the potential CO_2 release from tundra soils in a changing climate. *Applied Soil Ecology*. 11:127-134.

Cicerone, R.J & Oremland, R.S. 1988. Biogeochemical aspects of atmospheric methane. *Global Biogeochem.Cycles*. 2: 299–327.

Cicerone, R.J. & Shetter J.D. 1981. Sources of atmospheric methane:measurements in rice paddies and a discussion .*Journal of Geophysical Research*. 86: 7203-7209.

Cicerone, R.J., Shetter, J.D. and Delwiche, C.C.1983. Seasonal variations of methane flux from a California rice paddy. *J Geophys Res.*88:7203–7209.

Clymo, R. S. & Pearce, D. M. E. 1995. Methane and carbondioxide production in, transport through, and efflux from a peatland. Philos. Trans. R. Soc. Lond. A Math. Phys. Eng. Sci. 351(1696): 249–259.

Clymo, R. S. 1984. The limits to peat bog growth. Philos. Trans. R Soc. Lond. B Biol. Sci. 303(1117): 605–654.

Clymo, R. S., Turunen, J., & Tolonen, K. 1998. Carbon accumulation in peatland. *Oikos* 81(2): 368–373.

Clymo, R.S. 1987. The ecology of peatlands. *Science Progress Oxford* 71: 593–614.

Conrad, R. 1989.Control of methane emission from in terrestrial ecosystems.In Exchange of trace gases between terrestrial ecosystems and the atmosphere.(Edited by M.O.Andrea &D.S.Schimel). John Wiley and Sons. Chichester

Conrad, R. 1999. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments [review]. *FEMS Microbiology Ecology,* 28: 193–202.

Conrad, R., Schuëtz, H. and Babbel, M. 1987. Temperature limitation of hydrogen turnover and methanogenesis in anoxic paddy soil. *FEMS Microbiol. Ecol.* 45, 281-289.

Cowardin, L.M., Carter,V., Goelet,E.C. & LaRoe,E.T.1979. Classification of wetlands and deepwater habitats of the United States. USDI Fish and Wildlife Service,FWS/OBS-79/31.

Crill, P.M., Bartlett, K.B., Harris, R.C., Gorham, E., Verry, E.S., Sebacher, D.I., Madzar, I., & Sanner, W., 1988. Methane flux from Minnesota peatlands. *Global Biogeochemical Cycles* 2: 371–384.

Crill, P.M., Martikainen, P.J., Nykanen, H. & Silvola, J., 1994. Temperature and N fertilization effects on methane oxidation in a drained peatland soil. *Soil Biology & Biochemistry* 26: 1331–1339.

Crozier, C. R. , Devai, I & DeLaune, R. D. 1995. Methane and reduced sulfer gas production by fresh and dried wetland soils. *Soil Sci Soc. Am.J.* 59: 277-284.

Dacey, J.W.H. 1981a. Pressurized ventilation in the yellow water lily. *Ecology.* 62: 1137–1147.

Dacey, J.W.H. 1981b. How aquatic plants ventilate. *Oceanus* 24: 43–51.

Dannenberg, S., & Conrad, R., 1999. Effect of rice plants on methane production and rhizospheric metabolism in paddy soil. *Biogeochemistry.* 45: 53–71.

Daulat, W.E. & Clymo, R.S., 1998. Effects of temperature and water table on the efflux of methane from peatland surface cores. *Atmospheric Environment.* 32: 3207–3218.

De Bont, J.A.M., Lee, K.K. & D.F. Bouldin. 1978. Bacterial oxidation of methane in a rice paddy. *Ecol. Bull.* 26:91–96.

De Laune, R.D., Reddy, C.N. & Patrick, W.H.Jr. 1981.Accumulation of Plant Nutrients and Heavy Metals through Sedimentation Processes and Accretion in a Louisiana Salt Marsh. *Estuaries.* 4(4): 328-334.

De Laune, R.D., Smith, C.J. & Patrick, W.H. 1983.Methane release from Gulf coast wetlands. *Tellus.* 35:8-15.

Delphin, J.E. 1988. Use of first-order kinetics describing the mineralization in some cultivated soils. *Agronomie* 8: 289–294.

De Nobili, M., Contin, M., Mondini, C. & Brookes, P.C. 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biology & Biochemistry* 33: 1163–1170.

Denier van der Gon HAC, van Breemen N. 1993. Diffusion-controlled transport of methane from soil to atmosphere as mediated by rice plants. *Biogeochemistry.* 21:177-190.

Denier, van der Gon & Neue, H.U. H.A.C.,, 1996. Oxidation of methane in the rhizosphere of rice plants. *Biology and Fertility of Soils.* 22: 359–366.

Dickinson, R. E. & Cicerone, R. J. 1986. Future Global Warming from Atmospheric Trace Gases. *Nature* 319: 109-15.

Ding, W.X., Cai, Z.C., Tsuruta, H., 2004. Diel variation in methane emissions from the stands of Carex lasiocarpa and Deyeuxia angustifolia in a cool temperate freshwater marsh. *Atmospheric Environment.* 38:181–188

Ding, W., Cai, Z., Tsuruta, H.& Li, X., 2003. Key factors affecting spatial variation of methane emissions from freshwater marshes. *Chemosphere* 51: 167–173.Ding, W.X., Cai, Z.C. & Wang, D.X., 2004. Preliminary budget of methane emissions from natural wetlands in China. *Atmospheric Environment* 38: 751–759.

Ding, W.X., Cai, Z.C., Tsuruta, H.& Li, X.P., 2002. Effect of standing water depth on methane emissions from freshwater marshes in northeast China. *Atmospheric Environment* 36: 5149–5157.

Dise, N.B, 1993. Methane emission from Minnesota peatlands: Spatial and seasonal variability. *Global Biogeochem. Cycles* 7: 123–142

Dise, N.B.1992.Winter fluxes of methane from Minnesota peatlands. *Biogeochemistry.* 17(2).71-83.

Dlugokencky, E. 2001, NOAA CMDL Carbon Cycle Greenhouse Gases, Global average atmospheric methane mixing ratios, NOAA CMDL cooperative air sampling network, Http://www.cmdl.noaa.gov/ccg/ figures/ch4trend global.gif.

Dowrick, D.J.,Freeman,C., Lock,M.A. & Reynold,B. 2006. Sulphate reduction and the suppression of peatland methane emissions following summer drought. Geoderma.132(3-4):384-390.

Duan, X.N., Wang, X.K., Mu, Y.J. & Quyang, Z.Y. 2005. Seasonal and diurnal variations in methane emissions from Wuliangsu Lake in arid regions of China. *Atmospheric Environment.* 39:4479–4487.

Dunfield, P., Knowles, R., Dumont, R.& Moore, T.R., 1993. Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH. *Soil Biology & Biochemistry* 25: 321–326.

Edwards, C., Hales, B.A., Hall, G.H., Mc Donald, I.R., Murrell, J.C., Pickup, R.,Ritchie, D.A., Saunders, J.R., Simon, B.M. & Upton,M. 1998. Microbiological processes in the terrestrial carbon cycle: methane cycling in peat, *Atmospheric Environment.*32: 3247-3255.

Elferink, J.W.H.S., Visser, A., Hulshoff Pol L.W. & Stams, A.J.M. 1994. Sulfate reduction in methanogenic bioreactors, *FEMS Microbiol. Rev.* 15:119–136.

Falz, K.Z., Holliger, C., Grosskopf, R., Liesack, W., Nozhevnikova, A.N. & Muller, B. 1999. Vertical distribution of methanogens in the anoxic sediment of Rotsee (Switzerland). *Appl. Environ. Microbiol.* 65: 2402–2408.

Fechner-Levy, E.& Hemond, H.F., 1996. Trapped methane volume and potential effects on methane ebullition in a northern peatland. *Limnol. Oceanogr.* 41: 1375–1383.

Fey, A. & Conrad, R. 2000. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl. Environ. Microbiol.* 66: 4790–4797.

Fey, A Chin, K. J. & Conrad, R. 2001.Thermophillic methanogens in rice field soil. Environmental Microbiology. 3(5):295-303.

Freeman, C., Gresswell, R., Guasch, H., Hudson, J., Lock, M.A., Reynolds, B., Sabater, F. & Sabater, S. 1994a. The role of drought in the impact of climatic change on the microbiota of peatland streams. *Freshwat. Biol.* 32: 223–230.

Freeman, C., Lock, M.A. & Reynolds, B. 1993c. Fluxes of carbon dioxide, methane and nitrous oxide from a Welsh peatland following simulation of water table draw-down: Potential feed-back to climatic change. *Biogeochemistry* 19: 51–60

Frenzel, P & Bosse, U. 1996. Methyl fluoride, an inhibitor of methane oxidation and production. FEMS Microbiology and Ecology. 21: 25-36.

Frenzel, P. & Rudolph, J.1998. Methane emission from a wetland plant: the role of CH4 oxidation in Eriophorum. *Plant and Soil.* 202: 27–32.

Frenzel, P. 2000. Plant associated methane oxidation in rice fields and wetlands. In: Schink B.(ed.), Microbial Ecology. Kluwer, New York. 16:85-114.

Frye, J.P., Mills, A.L. and Odum,W.E. 1994. Methane flux in *Peltandra virginica* (*Araceae*) wetlands: comparison of field data with a mathematical model. *Am. J. Bot.* 81: 407–413.

Galand, P. E., Fritze, H. & Yrjaela, K. 2003. Microsite-dependent changes in methanogenic populations in a boreal oligotrophic fen. *Environ. Microbiol.*5:1133–1143.

Galand, P. E., Juottonen, H., Fritze, H. & K. Yrjaela. 2005. Methanogen communities in a drained bog: effect of ash fertilization. Microb. Ecol. 7(10) 1547-1557.

Galand, P. E., Saarnio, S., Fritze, H. & Yrjaela, K. 2002. Depth related diversity of methanogen Archaea in Finnish oligotrophic fen. *FEMS Microbiol. Ecol.* 42:441–449.

Garcia J.L. 1990.Taxonomy and ecology of methanogens. *FEMS Microbiol Rev.* 87: 297-308.

Garcia, J.L., Patel, B.K.C. & Ollivier, B. 2000. Taxonomic phylogenetic and ecological diversity of methanogenic Archaea. *Anaerobe* 6: 205-226.

Gerard, G. & Chanton, C.1993. Quantification of methane oxidation in the rhizosphere of emergent aquatic macrophytes: defining upper limits. *Biogeochemistry.*23(2): 79-97.

Gilbert, B. & Frenzel, P. 1995. Methanotrophic bacteria in the rhizosphere of rice microcosms and their effect on porewater methane concentration and methane emission. *Biology and Fertility of Soils.* 14:84-89.

Glissmann, K., Chin, K.J., Casper, P. & Conrad, R. 2004. Methanogenic pathway and archaeal community structure in the sediment of eutrophic Lake Dagow: effect of temperature. *Microbial Ecology* 48: 389–399.

Gransee, A. & Whittenmayer, L. 2000. Qualitative and quantitative analysis of water soluble root exudates in relation to plant species and development. *Journal of Plant Nutrition and Soil Science.* 163:381-385.

Greenup, A.G., Bradford, M.A., McNamara, N.M., Ineson, P.& Lee, J. 2000. The role of Eriophorum vaginatum in CH4 flux from an ombrotrophic peatland. *Plant and Soil* .227: 265–272.

Goodwin, K.A., Schaeffer, J.K. & Oremland, R.S. 1998. Bacterial oxidation of dibromomethane and methyl bromide in natural waters and enrichment cultures, *Appl. Environ. Microbiol.*, 64: 4629-4636.

Gopal, B., Kvet,J., Loffler, H., Masing, V & Patten,B.1979. Definition and classification. In Wetlands and Shallow Continental Water Bodies, Vol.1. Natural and Human Relationships, ed. B.C. Pattten, pp9-15. The Hague. The Netherlands:SPB Academic Publishing.

Grosse, W. & Mevi-Schutz, J. 1987. A beneficial gas-transport system in *Nymphoides peltata. Am.J.Bot.*,74:947-952.

Grosskopf, R., Janssen, P.H. & Liesack, W. 1998. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl. Environ. Microbiol.* 64: 960–969.

Grünfeld, S. & Brix, H., 1998. Methanogenesis and methane emissions, effects of water table, substrate type and presence of *Phragmites australis. Aquatic Botany.* 64: 63–75.

Hanson, R.S. & Hanson, T. E. 1996. Methanotrophic bacteria. Microbiological Reviews.60:439-471.

Happell, J., Chanton, J.P. & Showers, W. 1995. Methane transfer across the water-air interface in stagnant wooded swamps of Florida. *Limnology and Oceanography.* 40: 290–298.

Heathwaite, A.L. 1990. The effect of drainage on nutrient release from fen peat and its implications for water quality; a laboratory simulation. *Wat. Air Soil Polln.* 49: 159–173

Heiri, O., Lotter, A.F & Lemcke. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. G.*Journal of Paleolimnology* 25: 101–110, 2001.

Heyer, J., Berger, U., Kuzin, I.L. & Yakovlev, O.N., 2002. Methane emissions from different ecosystem structures of the subarctic tundra in Western Siberia during midsummer and during the thawing period. *Tellus 54B:.* 231–249.

Hirota, M., Tang, Y., Hu, Q., Hirata, S., Katoa, T., Moe,W.,Cao G. & Mariko. 2004. Methane emissions from different vegetation zones in a Qinghai-Tibetan Plateau wetland. *Soil Biology & Biochemistry* 36:737–748.

Hogg, E.H., Lieffers, V.J., &Wein, R.W. 1992. Potential carbon losses from peat profiles—effects of temperature, drought cycles, and fire. *Ecol. Appl.* 2 (3): 298–306.

Hoj, L., Olsen, R.A. & Torsvik, V.L. 2005.Archaeal communities in High Arctic wetlands at Spitsbergen, Norway (78°N) as characterized by 16S rRNA gene fingerprinting.2005. *FEMS Microbiol. Ecol.* 53:89–101.

Holzapfel-Pschorn A., Conrad, R. & Seiler, W. 1985. Production, oxidation and emission of methane in rice paddies. *FEMS Microbial. Ecol.* 31: 343-335.

Holzapfel-Pschorn A., Conrad, R. and Seiler, W. 1986. Effects of vegetation on the emission of methane from submerged paddy soil. *Plant Soil.* 92: 223-233.

Horn, M. A., Matthies, C., Kuessel, K., Schramm, A. & Drake, H. L. 2003. Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat. *Appl. Environ. Microbiol.* 69:74–83.

Hornibrook, E.R.C., Longstaffe, W.S. & Fyfe, W.S., 1997. Spatial distribution of microbial methane production pathways in temperate zone wetland soils: stable carbon and hydrogen isotope evidence. *Geochimica et Cosmochimica Acta* 61: 745–753.

Houghton JT, Jenkins GJ & Ephraum JJ.1990. Climate change. *The IPCC Scientific Assessment.* Cambridge University Press, Cambridge

Hyvoenen, T., Ojala, A., Kankaala, P.& Martikainen, P. 1998. Methane release from stands of water horsetail (*Equisetum fluviatile*) in a boreal lake. *Freshwater Biol.* 40: 275–284.

Ingvorsen, K. & Joergensen, B.B.1984. Kinetics of sulfate uptake by freshwater and marine species of *Desulfovibrio. Archives of Microbiology.*139(1):61-66.

IPCC (Intergovernmental Panel on Climate Change) 2001. Climate change 2001: the scientific basis. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, S., Maskell, K., Johnson, C.A. (Eds.), Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK; New York, NY, 881 pp.

IPCC (Intergovernmental Panel on Climate Change). 1994. Climate change:radiative forcing of climate change and an evaluation of the IPCC IS92 emission scenarios(Ed. J.T Houghton et.al). Cambridge University Press, Cambridge. UK, 1-339.

IPCC (Intergovernmental Panel on Climate Change) 1995. In: Houghton, J.T., Meira Filho, L.G., Bruce, J., Lee, H., Callander, B.A., Haites, E., Harris, N., Maskell, K. (Eds.), Climate change 1994, Radiative forcing of climate change and an evaluation of the IPCC IS92 Emission Scenarios, Cambridge Univ. Press, New York, USA.

Jackson, M.L.1958. Soil chemical analysis.Prentice-Hall of India Private Limited. New Delhi.

Jakobsen, P., Patrick, W.H. Jr, Williams, B.G. 1981. Sulfate and methane formation in soils and sediments. *Soil Sci* 123 :279–287

Jauhiainen, J., Takahashi, H., Heikkinen, J.E.P., Martikainen, P.J. & Vasander, H. 2005. Carbon fluxes from a tropical peat swamp forest floor. *Global Change Biology.* 2005.

Jetten, M. S.M., Stams, A. J.M. & Zehnder A.J.B. 1990. Acetate threshold values and acetate activating enzymes in methanogenic bacteria. *FEMS Microbiology Letters.* 73 (4): 339–344

Jetten, M. S.M., Stams, A. J.M. & Zehnder A.J.B.1992. Methanogenesis from acetate: a comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp. *FEMS Microbiology Letters* 88 (3-4): 181–198.

Joabsson, A. & Christensen, T.R., 2001. Methane emissions from wetlands and their relationship with vascular plants: an Arctic example. *Global Change Biology* 7: 919–932.

Joabsson, A., Christensen, T.R. & Wallen, B., 1999. Vascular plant controls on methane emissions from northern peatforming wetlands. *Trends in Ecology and Evolution.* 14: 385–388.

Jones, D.L. 1998. Organic acids in the rhizosphere—a critical review. *Plant and Soil* 205: 25–44.

Jones, D.L., Hodge, A. & Kuzyakov, Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* 163: 459–480.

Jones, D.L., Kemmitt, S.J., Wright, D., Cuttle, S.P., Bol, R.& Edwards, A.C., 2005. Rapid intrinsic rates of amino acid biodegradation in soils are unaffected by agricultural management strategy. *Soil Biology and Biochemistry* 37: 1267–1275.

Juutinen, S., Alm, J., Larmola, T., Huttunen, J., Morero, M., Saarnio, S., Martikainen, P.J., Silvola, J., 2003. Methane (CH_4) release from littoral wetlands of Boreal lakes during an extended flooding period. *Global Change Biology* 9: 413–424.

Juutinen, S., Alm, J., Martikainen, P., Silvola, J., 2001. Effects of spring flood and water level draw-down on methane dynamics in the littoral zone of boreal lakes. Freshwater Biology.46:855-869.

Kaeki, T., Ojala, A., Kankaala, P., 2001. Diel variation in methane emissions from stands of Phragmites australis (Cav.) Trin. Ex Steud. and Typha latifolia L. in a boreal lake. *Aquaic. Botany.* 71: 259–271.

Kelker, D. & Chanton, J., 1997. The effects of clipping on methane emissions from Carex. *Biogeochemistry.* 39: 37–44.

Kelley, C.A., Dise, N. & Martens, C.S. 1992. Temporal variations in the stable carbon isotopic composition of methane emitted from Minnesota peatlands. *Global Biogeochemical Cycles* 6: 263–269.

Kelley, C.A., Martens, C.S. & Ussler, W., 1995. Methane dynamics across a tidally flooded riverbank margin. *Limnology and Oceanography* 40: 1112–1129.

Kelly,C.A. & Chynoweth, D.P.1980. Comparison of in situ and in vitro rates of methane release in freshwater sediments. *Appl Environ Microbiol.* 40(2): 287-293.

Kelly, C.A & Chynoweth, D.P. 1981. The contributions of temperature and of the input of organic matter in controlling rates of sediment methanogenesis. *Limnol. Oceanogr.* 26(5): 1981, 891-897.

Kemnitz, D., Chin, K.J., Bodelier, P. & Conrad, R. 2004. Community analysis of methanogenic Archaea within a riparian flooding gradient. *Environmental Microbiology.* 6 : 449–461.

Kettunen, A., 2003. Connecting methane fluxes to vegetation cover and water table fluctuations at a micro site level: a modeling study. *Global Biogeochemical Cycles* 17: 2001–2019.

Kevbrin, V.V., Lysenko, A.M. W. & Zhilina T.N. 1997. Physiology of the alkaliphilic methanogen Z-7936, a new strain of Methanosalsus zhilinaeae isolated from Lake Magadi. *Microbiology Engl Tr* 66: 261-266

Khalil, M.A.K., 2000. Atmospheric methane: an introduction. In: Khalil, M., (Ed.), Atmospheric Methane: Its Role in the Global Environment, Springer, New York, pp. 1–8.

Kim J., Verma S.B. & Billesbach D.P. 1999. Seasonal variation in methane emission from a temperate *Phragmites*-dominated marsh: effect of growth stage and plant-mediated transport. *Global Change Biology* 5(4): 433–440.

Kim, J., Verma S.B. & Billesbach, D.P. 1998b. Seasonal variaton in methane emission from a temperate *Phragmites*-dominated marsh: effect of growth stage and plant-mediated transport. *Global Change Biol.* 5: 433–440.

Kim, J., Verma S.B., Billesbach, D.P. & Clement, R.J. 1998a. Diel variation in methane emission from a midlatitude prairie wetland: Significance of convective through-flow. *J. Geophys. Res.* 103: 28029– 28039.

Kimura, M., Miura, Y., Watanabe, A., Katoh, T. & Haraguchi, H. 1991. Methane emission from paddy field (Part 1) Effect of fertilization, growth stage and midsummer drainage: Pot experiment. *Environ Sci* 4: 265–271.

Kimura, G., Silver E. and Blum P. 1997. Proc. ODP, Init. Repts. vol. 170, Ocean Drilling Program, College Station, TX 458 pp.

King, G.M., 1990a. Regulation by light of methane emissions from a wetland. *Nature* 345:513–515.

King, G.M., Roslev, P. and Skovgaard, H. 1990. Distribution and rate of methane oxidation in sediments of the Florida Everglades. *Applied and Environmental Microbiology*.56:2902-2911.

King, G.M., 1994. Associations of methanotrophic bacteria with andmethane consumption by the roots of aquatic macrophytes. *Appl. Environ. Microbiol*.60 : 3220–3227.

King, J.Y., Reeburgh, W.S. & Regli, S.K. 1998. Methane emissions and transport by arctic sedges in Alaska: results of a vegetation removal experiment. *Journal of Geophysical Research.* 103: 29083–29092.

Klinger, L.F., Zimmerman, P.R., Greenberg, J.P., Heidt, L.E. & Guenther, A.B. 1994. Carbon trace gas fluxes along a successional gradient in the Hudson Bay lowland. *J. Geophys.Res.* 99 (D1): 1469–1494.

Kluëber, H. D. & Conrad R. 1998. Effects of nitrate, nitrite, NO and N2O on methanogenesis and other redox processes in anoxic rice ¢eld soil. *FEMS Microbiology Ecology* 25:301-318.

Knapp, A.K. & Yavitt J.B. 1992. Evaluation of a closed-chamber method for estimating methane emission from aquatic plants.*Tellus*.44B: 63-71.

Knapp, A.K. and Yavitt, J.B., 1995. Gas exchange characteristics of *Typha latifolia* L. from nine sites across North America. *Aquatic Botany*. 49: 203–215.

Koncalova, H. 1990. Anatomical adaptations to waterlogging in roots of wetland graminoids: limitations and drawbacks. *Aquatic Botany*.38:127-134.

Kotelnikova, S.V., Obraztsova, A.Y., Gongadze, G.M. & Laurinavichius, K.S. 1993. Methanobacterium thermoflexum sp. nov. and Methanobacterium defluvii sp. nov., thermophilic rod-shaped methanogens isolated from anaerobic digestor sludge. *Syst. Appl. Microbiol.* 16: 427- 435

Kotsyurbenko, O.R., Chin, K.J., Glagolev, M.V., Stubner, S., Simankova, M.V., Nozhevnokova, A.N. & Conrd, R. 2004. Acetoclastic and hydrogenotropic

methane production and methanogenic populations in an acidic West-Siberian peat bog.*Environ Microbiol* 6: 1159-1173.

Koyama, T. 1963. Gaseous metabolism in lake sediments and paddy soils and the production of atmospheric methane and hydrogen. *J. Geophys. Res.* 68: 3971–3973.

Kraffczyk, I., Trolldenier, G. & Beringer, H., 1984. Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. *Soil Biology and Biochemistry* 16: 315–322.

Kristjansson, J.K., Schoenheit, P., Thauer, R.K., 1982. Different Ks values for hydrogen of methanogenic bacteria and sulphate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. *Arch. Microbiol.* 131, 278– 282.

Kulshreshtha, K., Singh, S. N.& Agnihotri, S. 2000. Existence of micropores on the tiller surface of *Schoenoplectus subalatus*: the probable port for methane emission. *Aquatic Botany* 66: 241-247.

Kuzyakov, Y.& Domanski, G., 2000. Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science* 163: 421–431.

Kuzyakov, Y. & Bol, R. 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biology and Biochemistry 38 (4): 747-758.

Lansdown, J. M., Quay, P. D. and King, S. L. 1992. CH_4 production via CO_2 reduction in a temperate bog: a source of 13-C-depleted CH_4. *Geochim. Cosmochim. Acta.* 56:3493–3503.

Lansdown, J.M., Quay, P.D. & King, S.L. 1992. CH_4 production via CO_2 reduction in a temperate bog: A source of ^{13}C-depleted CH_4. *Geochim. Cosmochim. Acta.* 56: 3493–3503.

Lelieveld, J., Crutzen, P.J. & Dentener, F.J. 1998. Changing concentrations, lifetime and climate forcing of atmospheric methane. *Tellus, 50B*: 128–150.

Lindau, C. W. 1994. Methane emissions from Louisiana rice fields amended with nitrogen ferterlizers. *Soil Biol. Biochem.* 26, 353–359.

Liu Y., Boone D.R. & Choy C. 1990. Methanohalophilus oregonense sp. nov., a methylotrophic methanogen from an alkaline, saline aquifer. *Int J Syst Bacteriol* 40: 111-116

Lovley, D.R. & Phillips, E.J.P. 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl Environ Microbiol* 51: 683–689.

Lovley, D.R. 1985. Minimum threshold for hydrogen metabolism in methanogenic bacteria. *Appl. Environ. Microbiol.* 49: 1530-1531.

M., Harder W. & Schleifer K.-H. (eds), The Prokaryotes, 2nd ed. pp. 719-767. Springer-Verlag, Berlin

McDonald, I.R., Upton, M., Hall,G., Pickup, R.W., Edwards, C., Saunders,J.R., Ritchie, D,A. & Murrell, J.C. 1999. Molecular Ecological Analysis of Methanogens and Methanotrophs in Blanket Bog Peat. *Microbial Ecology.* 38:225-233.

MacDonald J.A., Fowler D., Hargreaves K.J., Skiba U., Leith I.D. and Murray M.B. 1998. Methane emission rates from a northern wetland; Response to temperature, water table and transport. *Atmospheric Environment.* 32(19): 3219–3227.

MacDonald,J.A.,Skiba,U.M.,Shepad,L.J.,Harggreaves,K.J.,Fowler,D & Smith,K.A.1996. Soil environmental variables affecting CH_4 flux from a range of forest, moorland and agricultural soils. Biogeochemistry. 34:113-132.

Madigan, M.T., Martinko, J.M. & J, Parker. 2003. Brock: Biology of Microorganisms, [10th ed.]. Prentice-Hall.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd edn. Academic Press, London.

Martikainen, P.J., Nykaenen, H., Alm, J.1995. Change in fluxes of carbon dioxide, methane and nitrous oxide due to forest drainage of mire sites of different trophy. *Plant and Soil.* 168–169, 571–577.

Mathrani I.M., Boone D.R., Mah R.A., Fox G.E. & Lau, P.P. 1988. Methanohalophilus zhilinae sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. *Int J Syst Bacteriol* 38: 139-142

Matthews, E. & Fung, I. 1987. Methane emission from natural wetlands: global distribution, area, and environmental characteristics of sources. *Global Biogeochemical Cycles.* 1: 61–86.

Matthews, R.B. & Wassmann, R.2003. Modelling the impacts of climate change and methane emission reductions on rice production: a review. *European Journal of Agronomy,* 19: 573-598.

Mayer, H.P. & Conrad, R. 1990. Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. *FEMS Microbiology Ecology*, 73: 103–112.

McCartney, D.M. & Oleszkiewizc, J.A., 1993. Competition between methanogens and sulfate reducers: effect of COD:sulfate ratio and acclimation, *Water Env. Res.* 65: 655-664.

Mckane, R. B., E. B. Rastetter, J. R. Melillo, G. R. Shaver, C. G. Hopkinson, D. N. Fernandes, D. L. Skole, & Ciiomentowski, W. H.. 1995. Effects of global change on carbon storage in tropical forests of South America. *Global Biogeochem. Cycles* 9: 329-350.

Metje, M. & Frenzel, P. 2005. Effect of temperature on anaerobic ethanol oxidation and methanogenesis in acidic peat from a northern wetland. *Appl. Environ.Microbiol.* 71 : 8191–8200

Middelburg, J.J., Klaver, G., Vlug, J., Wielemaker, A., Haas, W. van der Nat,J.F.W.A. 1996.Organic matter mineralization in intertidal

Mikkela, C., Sundh, I. & Svensson, B.H.1995. Diurnal variation in methane emission in relation to the water table, soil temperature, climate and vegetation cover in a Swedish acid mire. *Biogeochemistry.* 28: 93–114.

Minoda, T., & Kimura, M. 1994. Contribution of photosynthetic carbon to the methane emitted from paddy fields. *Geophys. Res. Lett.* 21:2007–2010.

Mitsch, W.J. & Gooselink, J. G . 2000. Wetlands. John Wiley & Sons. Canada.

Moore, R & Knowles, R. 1990. Methane emissions from fen, bog and swamp peatlands in Quebec. *Biogeochemistry.* 11(1):45-61.

Moore, T.R. & Knowles, R. 1989. The influence of water table levels on methane and carbon dioxide emissions from peatland soils. *Canadian Journal of Soil Science.* 69. 33–38.

Moore, T.R. & Knowles, R.1987. Methane and carbon dioxide evolution from subarctic fens. *Canadian Journal of Soil Science.* 67: 77–81

Moore, T.R., Roulet, N.T. & Waddington, J.M. 1998. Uncertainties in predicting the effect of climatic change on the carbon cycling of Canadian peatlands. *Climatic Change*, 40, 229–245.

Moore, T,. R. & Roulet, N. T. 1993. Methane flux:water table relations in northern wetlands. *Geophysical Research Letters.* 20:587-590.

Morrisey, L. A. , Zobel, D.B. & Livingston G. P. 1993. Significance of stomatal control onmethane release from Carex-dominated wetlands.In: Hutzinger O (ed.) *Chemosphere* 26: 339-355.Pergamon Press Ltd., Oxford.

Mosier, A.R. & Bouwman, A.F. 1993. Working group report: Nitrous oxide emissions from agricultural soils? in A.R. van Amstel (ed.), *Methane and Nitrous Oxide: Methods in National Emission Inventories and Options for Control Proceedings*, The Netherlands, National Institute of Public Health and Environmental Protection, Bilthoven, pp. 343-6.

Mosier, A.R., Wassman,R., Verchot, L., King, J. & Palm, C. 2004. Methane and Nitrogen Oxide Fluxes in Tropical Agricultural Soils: Sources, Sinks and MechanismsMethane and Nitrogen Oxide Fluxes in Tropical Agricultural Soils: Sources, Sinks and Mechanisms. *Environment,Development and Ssustainibility.* 6(1-2): 11-49.

Mukhopadhyay SK, Biswas H, Das KL et al. 2001. Diurnal variation of carbon dioxide and methane exchange above Sundarbans mangrove forest, in NW coast of India. *Indian Journal of Marine Sciences*. 30: 70–74.

Mukhopadhyay, S.K., Biswas, H. & De, T.K. 2002. Impact of Sundarban mangrove biosphere on the carbon dioxide and methane mixing ratios at the NE coast of Bay of Bengal, India. *Atmospheric Environment.* 36, 629–638.

Murase, J. & Sugimoto, A. 2001. Spatial distribution of methane in the Lake Biwa sediments and its carbon isotopic compositions. *Geochem J.*35:257–263

Murase, J. & Sugimoto, A. 2002. Seasonal and spatial variations of methane production in mesotrophic lake sediments (Lake Biwa, Japan). *Verh Int Verein Limnol* 28:971–974

Nakagawa,F., Yoshida,N., Nojiri,Y. & Makarov, V.N.2002. Production of methane from alasses in eastern Siberia: Implications from its 14C and stable isotopic compositions. *Global Biogeochemical Cycles*.16:3,14-1-15.

Nedwell D.B. & Watson A. 1995. Methane production, oxidation and emission in a U.K. ombrotrophic peat bog: influence of SO_4^{2-} from acid rain. *Soil Biology and Biochemistry*. 27:893-903.

Nouchi, I. & Mariko, S. 1993. Mechanism of methane transports by rice plants. In Biogeochemistry of Global Change. Radiatively Active Trace Gases. (Ed.R. S. Oremland) Chapman & Hall, New York. Pp. 336-352.

Nouchi, I., Mariko S., & K. Aoki. 1990. Mechanism of methane transport from the rizosphere through rice plants. Plant Physiol. 94: 59-66.

Nuesslein, B.,Chin, K.J., Eckert, W. & Conrad,R. 2001. Evidence for anaerobic syntrophic acetate oxidation during methane production in the profundal sediment of subtropical Lake Kinneret (Israel). *Environmental Microbiology*. 3(7):460-470.

Nyakanen, H., Alm, J., Lang, K., Silvola, T.& Martikainen, P.J., 1995. Emissions of CH_4, N_2O and CO_2 from a virgin fen drained for grassland in Finland. *Journal of Biogeography* 22: 351–357.

Orawan,S. & Suphasuk, P. 2002. Reduction of methane emission from submerged paddy soils by water management in Thailand. Symposium No.7; Paper.No.7. 202.195.252.61/kc_htm/turangxue/shijieturangdahui/papers/1638.pdf

Otter,L.B. & Scoles, M.C. 2000. Methane sources and sinks in a periodically flooded South African savanna. *Global biogeochemical cycles*. 14:97-111.

Parashar, D.C., Mitra, A.P., Gupta, P.K., Rai, J., Sharma, R.C., Singh, N., Koul, S., Ray, H.S., Das, S.N., Parida, K.M., Rao, S.B., Kanungo, S.B., Ramasami, T., Nair , B.U., Swamy, M., Singh, G., Gupta, S.K., Singh, A.R., Saikia, B.K.,

Barua, A.K.S., Pathak, M.G., Iyer, C.S.P., Gopalakhrishnan, M., Sane, P.V., Singh, S.N., Banerjee, R., Sethunathan, N., Adhya, T.K., Rao, V.R., Palit, P., Saha, A.K., Purkait, N.N., Chaturvedi, G.S., Sen, S.P., Sen, M., Sarkar, B., Banik, A., Subbaraya, B.H., Lal, G., Chaudhary, A.& Sinha, S.K.1996. Methane budget from paddy fields in India. *Chemosphere*.33: 737-757.

Parashar, D.C., Mitra, A.P., Gupta, P.K., Rai, J., Sharma, R.C., Singh, N., Koul, S., Ray, H.S., Das, S.N., Parida, K.M., Rao, S.B., Kanungo, S.B., Ramasami, T., Nair, B.U., Swamy, M., Singh, G., Gupta, S.K., Singh, A.R., Saikia, B.K., Barua, A.K.S., Pathak, M.G., Iyer, C.S.P., Gopalakhrishnan, M., Sane, P.V., Singh, S.N., Banerjee, R., Sethunathan, N., Adhya, T.K., Rao, V.R., Palit, P., Saha, A.K., Purkait, N.N., Chaturvedi, G.S., Sen, S.P., Sen, M., Sarkar, B., Banik, A., Subbaraya, B.H., Lal, G., Chaudhary, A. & Sinha, S.K.1994. Methane budget from paddy fields in India. Current Science. 66. No.12: 938-941.

Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. *European Journal of Soil Science* 54: 741–750.

Patrick WH, Reddy CN (1978) Chemical changes in rice soils. In: Soils and rice. International Rice Research Institute, Manila, Philippines, pp 361–379

Pearman, G.I. & Fraser, G.J. 1988. Sources of increased methane. *Nature.*(Londn.) 332 :489-490.

Ponnamperuma, F.N. 1972. The chemistry of submerged soils. *Adv. Agron.* 24:29–96.

Popp, T.J., Chanton, J.P., Whiting, G.J. & Grant, N., 1999. TheMethane Stable Isotope Distribution at a Carex Dominated Fen In North Central Alberta. *Global Biogeochemical Cycles* 13:1063–1077.

Popp,T.J. & Chanton,J.P.1999. Methane stable isotope distribution at a *Carex* dominated fen in north Central Alberta. *Global Biogeochemical Cycles.* 13:1063-1077.

Priha, O. & Smolander, A., 2003. Short-term uptake of (NH_4 +)–N-15 into soil microbes and seedlings of pine, spruce and birch in potted soils. *Biology and Fertility of Soils* 37: 324–327.

Pulliam, W.M. & Meyer, J.L.1992. Methane emissions from floodplain swamps of the Ogeechee River: long-term patterns and effects of climate change. *Biogeochemistry*. 15(3):151-174

Purvaja, R., Ramesh, R & Frenzel, P. 2004. Plant-mediated methane emission from an Indian mangrove. *Global Change Biology*. 10: 1825–1834.

Purvaja., R. & Ramesh, R. 2001. Natural and anthropogenic methane emission from coastal wetlands of South India. *Environmental Management* 27: 547–557.

Raich, J. & Schlesingtk, W. H. 1992. The global carbon dioxide flux in soil respiration and relationship to vegetation and climate. *Tellus Ser. B* 44: 81-99.

Ramanathan, V., Cicerone, R., Singh, H., and Kiehl, J. 1985. Trace Gas Trends and Their Potential Role in Climate Change. *J. Geophysical Research* 90: 5547-66

Rejmankova E. and Post R.A. 1996. Methane in sulfate-rich and sulfate-poor wetland sediments. *Biogeochemistry*.34: 57–70.

Ritchie,D.A., Edwards, C., Mc Donald, I.F & Murrell, J.C.1997. Detection of methanogens and methanotrophs in natural environments. *Global Change Biology*. 3:339-350.

Robinson, J.A and Tiedje,J.M. 1984. Competition between sulfate-reducing and methanogenic bacteria for H2 under resting and growing conditions. *Arch. Microbiology*.137.26-32.

Roden, E.E. & Wetzel, R.G. 1996. Organic carbon oxidation and suppression of methane production by microbial Fe (III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. *Limnol Oceanog* 41: 1733–1748.

Roy, R. & Conrad, R. 1999. Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. *FEMS Microbiology Ecology* 28 (1): 49–61.

Saarnio, J. A., Silvola, J., Lohila, A., Nykänen, H. & Martikainen, P. J. Seasonal variation in CH_4 emissions and production and oxidation potentials at microsites on an oligotrophic pine fen. *Oecologia.* 110(3):414-422.

Sand-Jensen K, Prahl, C & Stokholm, H.1982. Oxygen release from roots of submerged aquatic macrophytes. *Oikos* 38: 349–354.

Sass, R. L., Fisher, F. M., Harcombe, P. A. & Turner, F. T. 1990. Methane Production and Emission in a Texas Agricultural Wetland. *Global Biogeochem. Cycles,* 4: 47-68.

Sass, R.L., & Fisher, F.M. 1995. Methane emissions from Texas rice fields: A five-year study. p. 46–59. *In* S. Peng, K.T. Ingram, H.-U. Neue, and L.H. Ziska (ed.) Climate change and rice. Springer-Verlag, Berlin.

Sass, R.L.,. Andrews, J.A., Ding, A & Fisher, F.M.Jr. 2002.Spatial and temporal variability in methane emissions from rice paddies: Implications for assessing regional methane budgets. *Nutrient Cycling in Agroecosystems.*64(1-2):3-7.

Saunder, D. H. & Grant, P.M. 1962. Rate of mineralization of organic matter in cultivated Rhodesian soils. New Zealand: International Soil Science Conference.

Schimel, J., 1995. Plant transport and methane production as controls on methane flux from arctic wet meadow tundra. *Biogeochemistry.*28: 183–200.

Schink, B. 1992. syntrophism among prokaryotes. In: The Prokaryotes (Balows,A., Trueper, H.G., Dworkin, M.,Harder,W. and Schleifer,K.H.,Eds.2nd Edn.1:276-299.Springer, New York.

Schnuerer, A. G., Zellner, G. & Svensson. B. 1999. Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. *FEMS Microbiol. Ecol.* 29:249–261.

Scholten, J. C.M.,. van Bodegom, P. M. Vogelaar, J., van Ittersum, A., Hordijk, K., Roelofsen, W & Stams, A. J.M. 2002. Effect of sulfate and nitrate on acetate conversion by anaerobic microorganisms in a freshwater sediment *FEMS Microbiology Ecology* 42 (3): 375–385

Schulz, S., Matsuyama, H. & Conrad, R. 1997. Temperature dependence of methane production from different precursors in a profundal sediment (Lake Constance). *FEMS Microbiol. Ecol.* 22: 207–213.

Schutz H., Seiler W. and Conrad R. 1989. Processes involved in formation and emission of methane in rice paddies. *Biogeochemistry.* 7: 33–53.

Sculthorpe, C. D. 1985 The Biology of Aquatic Vascular Plants. Koeltz Scientific Books, Konigstein, Germany.

Sebacher, D.I., Harriss, R.C. & Bartlett K. B. 1985. Methane emissions to the atmosphere through aquatic plants. *J. Env.Quali.*14: 40-46.

Segers, R. 1998. Methane production and consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry.* 41: 23-51.

Seiler, W., Holzapfel-Pschorn, A., Conrad, R. & Scharffe, D. 1984. Methane emission from rice paddy. *J. Atmos. Chem.* 1: 241-268.

Seiler, W.1984. Contribution of biological processes to the global budget of methane in the atmosphere. In: Klug, M.J. & Reddy, C.A.(eds) Current perspectives in Microbial Ecology,pp468-477. American Society of Microbiology, Washington, DC, USA.

Shannon, R.D. & White, J.R., 1994. A three-year study of controls on methane emissions from two Michigan peatlands. *Biogeochemistry* 27:35–60.

Simard, R.R. & N'dayegamiye, A.1993. Nitrogen-mineralization potential of meadow soils. *Canadian. J. Soil Sciences./Rev. Can. Sci. Sol.* 73(1): 27-38.

Stotzky, G. 1965. Microbial respiration. p. 1550–1572. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Strom, L., Ekberg, A., Mastepanov, M. & Christensen, T. B. 2003. The effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. *Global Change Biol.* 9:1185–1192.

Sugimota, A. & Wada, E. 1995. Hydrogen isotopic composition of bacterial methane: CO_2/H_2 reduction and acetate fermentation. *Geochimica et Cosmochimica Acta* 59: 1329–1337.

Sundh, I., Nilsson, M., Granberg, G., Svensson, B.H., 1994. Depth distribution of microbial production and oxidation of methane in northern boreal peatlands. *Microbial Ecology* 27: 253-265.

Svensson, B.H. 1984. Different temperature optima for methane formation when enrichments from acid peat are sup-plemented with acetate or hydrogen. *Appl. Environ. Microbiol.* 48: 389-394

Svensson, H. & Rosswall, T.1984. In situ methane production from acid peat in plant communities with different moisture regimes in a subarctic mire. *Oikos*.43:341-350.

Thauer, R. K. 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson.*Microbiology*.144: 2377-2406.

Thiele, J.H. and Zeikus, J.G. 1988. Control of interspecies electron flows during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. *Appl. Environ. Microbiol.* 54, 20-29.

Thomas, K.L., Benstead, J., Davies, K.L. & Lloyd, D., 1996. Role of wetland plants in the diurnal control of CH4 and CO2 fluxes in peat. *Soil Biol. Biochem.* 28: 17–23.

Torn, M.S. & Chapin, F. S. 1993 Environmental and biotic controls over methane flux from arctic tundra. *Chemosphere* 26: 357–368.

Tornbjerg, T., Bendix, M. & Brix, H. 1994. Internal gas transport in Typha latifolia L. and Typha augustifolia L. 1. Convective through-flow pathways and ecological significance. *Aquatic Botany* 49: 91–105.

Trivedy,R.K. & Goel,P.K.1984. Chemical and biological methods for water pollution studies. Published by Environmental Publications,Karad.

Tsuyuzaki, S., Nakano,T., Kuniyoshi, S. & Fukuda, M.2001.Methane flux in grassy marshlands near Kolyama River, North-Eastern Siberia. *Soil Biology & Biochemistry* 33:1419-1423.

Upton, M., Hill, B. C. , Edwards, Saunders, J. R., Ritchie, D. A. & Lloyd, D. 2000. Combined molecular ecological and confocal laser scanning microscopic analysis of peat bog methanogen populations. *FEMS Microbiol. Lett.* 193:275–281.

Valentine, D. L. 2002. Biogeochemistry and microbial ecology of anaerobic methane oxidation: a review. *Antonie van Leewenhoek.* 81: 271-282.

Valentine, D.W., Holland, E.A. & Schimel, D.S. 1994. Ecosystem and physiological control over methane production in northern wetlands. *Journal of Geophysical Research* 99 (1): 1563–1571.

Van den Pol-van Dasselaar, A., van Beusichem, M.L. & Oenema, O. 1999. Determinants of spatial variability of methane emissions from wet grasslands on peat soil. *Biogeochemistry.*44: 221–237.

Van der Krift, T.A.J., Kuikman, P.J., Moller, F. & Berendse, F. 2001. Plant species and nutritional-mediated control over rhizodeposition and root decomposition. *Plant and Soil* 228:191–200.

Van der Nat, F.J.W.A. & Middelburg, J. J. 1998a. Effects of two common macrophytes on methane dynamics in freshwater sediments. *Biogeochemistry.* 43: 79-104.

Van der Nat, F.-J.W.A. & Middelburg, J.J., 1998b. Seasonal variation in methane oxidation by the rhizosphere of *Phragmites australis* and *Scirpus lacustris. Aquatic Botany* 61, 95–110.

Van der Nat, F.-J.W.A., Middelburg, J.J., vanMeteren, D.,Wielemakers, A. 1998. Diel methane emissions patterns from *Scirpus lacustris* and *Phragmites australis. Biogeochemistry.* 41: 1–22.

Van Kessel, J.A.S. & Russell, J.B. 1996. The effect of pH on ruminal methanogenesis. *FEMS Microbiology Ecology.* 20: 205–210.

Van Veen, J. A., Merckx, R. & Van de Geijn, S.C. 1989. Plant- and soil-related controls of the flow of carbon from roots through the soil microbial biomass. *Plant Soil* 115:179–188.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring biomass C. *Soil Biol. Biochem.* **19:**703–707.

Verma,A., Subramaniam, V.& Ramesh,R. 2002. Methane emissions from a coastal lagoon: Vembanad Lake, West Coast, India. Chemosphere. 47:883-889.

Wagner, D. & Pfeiffer, E.M. 1997. Two temperature optima of methane production in a typical soil of the Elbe river marshland. *FEMS Microbiol. Ecol.* 22:145-153.

Wallen, B. 1986 Above and below ground dry mass of three main vascular plants on hummocks on a subarctic peat bog. *Oikos* 46: 51–56.

Wang, W. C., Yung, Y. L., Lacis, A. A., Mo, T. & Hansen, J. E. 1976. Greenhouse effects due to man-made perturbations of trace gases. *Science* 194, 685–690.

Wang, J & Bakken, L.S. 1997. Competition for nitrogen during decomposition of plant residues in soil: effect of spatial placement of n-rich and n-poor plant residues. *Soil Biol Biochem.* 29(2): 153-162.

Wang, Z & Han,X. 2005. Diurnal variation in methane emissions in relation to plants and environmental variables in the Inner Mongolia marshes. *Atmospheric Environment* 39: 6295–6305.

Wang, Z.P., Zeng, D. & Patrick, W.H.Jr. 1997. Characteristics of methane oxidation in a flooded rice soil profile. *Nutrient Cycling in Agroecosystems.* 49:97-103.

Wang, Z.P. & Han, X.G., 2005. Diurnal variation in methane emissions in relation to plants and environmental variables in the inner Mongolia marshes. *Atmospheric Environment.* 39: 6295–6305.

Wang, Z.P., Delaune, R.D., Masscheleyn, P.H.& Patrick, W.H., 1993. Soil redox and pH effects on methane in a flooded rice soil. *Soil Science Society of America Journal* 57:382–385.

Wang, Z.R., De Laune, R.D., Lindau, C.W. and Patrick, W.H.Jr. 1992. Methane production from anaerobic soil amended with rice straw and nitrogen fertilizers. *Ferti. Res.*33:115-121.

Wardle, D.A. 1999. How food webs make plants grow. *Trends in Ecology and Evolution.* 14: 418–420.

Watson. A, Stephen, K. D, Nedwell, D. V & Arah, J. R. M. 1997. *Soil Biology and Biochemistry.* 29,8:1257-1267.

Watson A, Nedwell D. 1998. Methane production and emissions from peat: the influence of anions (sulfate, nitrate) from acid rain. *Atmospheric Environment* 32:3239–3245.

Watson, R.T., Meira, F., L.G., Sanhueza, E. and Janetos, A. 1992. Sources and sinks. In: Houghton, J.T., Callander, B.A. and Varney, S.K. (Eds.), Climate Change 1992: The Supplementary Report to The IPCC Scientific Assessment, Cambridge University Press, Cambridge, UK, pp. 25-46.

Watt Committee on Report Methane Emissions 1994.edns William A. Department of Trade and Industry Environment and Energy Technology Division.

Weber, S., Leuders, T., Friedrich,M.W. & Conrad, R. 2001. Methanogenic populations involved in the degradation of rice straw in anoxic paddy soil. *FEMS Microbiology Ecology* 38:11-20.

Whalen S.C. & Reeburg W.S.1990. A methane flux transect along the trans-Alaska pipeline haul road. *Tellus 42:* 237-249.

Whalen, M. 1993. The global methane cycle. *Annu Rev.Earth Planet Sci* 21: 407–426.

White, G. & Ganf,G. 2002. A comparison of the morphology, gas space anatomy and potential for internal aeration in *Phragmites australis* under variable and static water regimes. *Aquatic Botany.* 73:115–127.

White, S.D., Ganf, G.G., 2000a. Lacunal anatomy and resistance to convective flow in *Typha domingensis* and *Phragmites australis. Aquatic Botany.* 68: 165–177.

White, S.D., Ganf, G.G., 2000b. Flow characteristics and internal pressure profiles in leaves of *Typha domingensis. Aquatic Botany.* 67: 263–273.

Whiticar, M.J. 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem Geol* 161: 291–314.

Whiticar, M.J., Faber, E. & Schoell, M. 1986. Biogenic methane formation in marine an freshwater environments: CO_2 reduction vs. acetate fermentation-isotope evidence. *Geochim. Cosmochim. Acta.* 50: 693–709.

Whiticar, M.J., Faber, E. & Schoell, M., 1986. Biogenic methane formation in marine and freshwater environments: CO2 reduction vs. acetate fermentation - isotope evidence. *Geochimica et Cosmochimica Acta* 50: 693–709.

Whiting G.J. & Chanton, J. P. 1992. Plant- dependent CH_4 emission in a subarctic Canadian fen. *Global Biogeochemistry Cycles.*5 :225-231.

Whiting, G. J., Chanton, J.P., Bartlett, D.S. & Hapell, J.D. 1991. Relationships between methane emission, biomass, and carbon dioxide exchange in a subtropical grassland. *Journal of Geophysical Research.* 96: 13067-13071.

Whiting, G.J. & Chanton, J.P. 1993. Primary production control of methane emission from wetlands. *Nature.* 364: 794-795.

Whiting, G.J. & Chanton, J.P.,1996. Control of the diurnal pattern of methane emission from emergent aquatic macrophytes by gas transport mechanisms. *Aquatic Botany.* 54: 237–253.

Whitman, W B.;Bowen, T L.; Boone, D R. 1992. The methanogenic bacteria. In: Balows A, Trüper H G, Dworkin M, Harder W, Schleifer K H. , editors;

Balows A, Trüper H G, Dworkin M, Harder W, Schleifer K H. , editors. The prokaryotes. 2nd ed. New York, N.Y: Springer-Verlag; pp. 719–767.

Whitton, B. A. & Rother, J. A.1988. Environmental features of deepwater rice fields in Bangladesh during the flood season. In 1987 International Deepwater Rice Workshop .pp 47-54. International Rice Research Institute, Manila, Philippines.

Widdel, F Microbiology and ecology of sulfate-and sulfur-reducing bacteria - Biology of Anaerobic Microorganisms, 1988 - John Wiley & Sons.

Widdel, F. 1988. Microbiology and ecology of sulfate and sulfur reducing bacteria. In: Biology of Anaerobic Microorganisms (ed. A.J.B. Zehnder), pp. 469–587. John Wiley & Sons, New York.

Williams, R. T. & Crawford, R. L. 1984 Methane production in Minnesota peatlands. *Appl. Environ. Microbiol.* 47: 1266–1271.

Winfrey, M. R. & Zeikus, J.G. 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. *Appl Environ Microbiol.*; 33(2): 275-281.

Woese, C. R., L. J. Magrum, and G. E. Fox. 1978. Archaebacteria. *J. Mol. Evol.* 11:245-252.

Wu, X. L.,Chin, K.J. & Conrad, R.2002. Effect of temperature stress on structure and function of the methanogenic archaeal community in a rice field soil. *FEMS Microbiology Ecology*. 39:211-218.

Xiao, X., Boles, S. Frolking, S. Salas, W. Moore, B. Li, C. He, L.& R.Zhao, Observations of flooding and rice transplanting of paddy rice fields at the site to landscape scales in China using VEGETATION sensor data. *Int. J. Remote Sens*. 23, 3009–3022, 2002.

Xing, Y., Xiea, P., Yanga, H., Wua, A. & Nia, L. 2006. The change of gaseous carbon fluxes following the switch of dominant producers from macrophytes to algae in a shallow subtropical lake of China. *Atmospheric Environment* 40:8034–8043

Yagi, K. & Minami, K. 1990. Effect of organic matter application on methane emission from some Japanese paddy fields.*Soil Science and Plant Nutrition.* 36: 599-610.

Yamamoto S., Alcauskas J.B. & Crozier T.E. 1976. Soubility of methane in distilled water and seawater. *Journal of Chemical and Engineering Data.* 21: 78-80.

Yavitt, J. B.& Knapp, A. K. 1998 Aspects of methane flow from sediment through emergent cattail (*Typha latifolia*) plants. *New Phytol.* 139:495–503.

Yavitt, J.B. & Lang G.E. 1990. Methane production in contrasting wetland sites: Response to organicchemical components of peat and to sulfate reduction. *Geomicrobiol. J* 8: 27–46.

Yavitt, J.B. 1997. Methane and carbon dioxide dynamics in *Typha latifolia* (L.) wetlands in Central New York State. *Wetlands.* 17 (3): 394-406.

Yavitt, J.B., Williams, C.J., Wieder, R.K. 2005. Soil chemistry versus environmental controls on production of CH4 and CO2 in northern peatlands. *European journal of Soil Science* 56(2):169-178.

Zehnder, A. J. B. 1978. Ecology of methane formation. In R. Mitchell (ed.) Water Pollution Microbiology, Vol 2. John Wiley & Sons, New York pp. 349-376.

Zehnder, A.J.B., & Brock, T.D.. 1979. Methane formation and methane oxidation by methanogenic bacteria. *J. Bacteriol.* 137:420–432.

Zeikus, J.G. & Winfrey, M.R.1976. Temperature limitation of methanogenesis in Aquatic Sediments. *Appl. Environ. Microbiol.* 31 :99-106.

Zhang, J, Song, C & Yang, W. 2005. Cold season CH_4, CO_2 and N_2O fluxes from freshwater marshes in northeast China. *Chemosphere.* 59: 1703–1705.

Zinder, S.H. 1993. Physiological ecology of methanogens. In: Ferry, J.G.(editor).Methanogenesis: Ecology, Physiology, Biochemistry, and Genetics. New York: Chapman and Hall.

Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S. & White, D.C. 1997. Compositional and functional shifts in microbial communities due to soil warming. *Soil Sci Soc Am J.*61:475–485